FORMULATION, OPTIMIZATION AND EVALUATION OF SILYMARIN NANOSPONGES

A Dissertation submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI – 600 032

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY
IN
BRANCH- I -> PHARMACEUTICS

Submitted by

ANN MARIA SUNNY
REGISTRATION No: 261610151

Under the guidance of

Mrs. M.A AMUTHA GNANA ARASI, M. Pharm., (Ph.D.),
Department of Pharmaceutics

COLLEGE OF PHARMACY
SRI RAMAKRISHNA INSTITUTE OF PARAMEDICAL SCIENCES
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This is to certify that the M. Pharm dissertation entitled "**FORMULATION, OPTIMIZATION AND EVALUATION OF SILYMARIN NANOSPONGES**" being submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai was carried out by **ANN MARIA SUNNY (Register no: 261610151)** in the Department of Pharmaceutics, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under my direct supervision, guidance and to my fullest satisfaction.

Mrs. M. A. AMUTHA GNANA ARASI, M. Pharm.,(Ph.D.).,
Assistant Professor,
Department of Pharmaceutics,
College of Pharmacy,
SRIPMS,
Coimbatore – 641 044.

Place: Coimbatore
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Dr. M. GOPAL RAO, M. Pharm, Ph.D.,
Vice Principal & HOD,
Department of Pharmaceutics,
College of Pharmacy,
SRIPMS,
Coimbatore - 641 044.

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Dr. T.K. RAVI, M. Pharm, Ph.D., FAGE.
Principal,
College of Pharmacy,
SRIPMS
Coimbatore -641 044.

Place: Coimbatore
Date:

ACKNOWLEDGEMENT

I thank **God Almighty** for the wisdom and perseverance that had been bestowed upon me during this research project. It's because of His grace I was able to successfully complete my project.

Words are insufficient to express my gratitude and respect towards my guide, **Mrs. M.A. Amutha Gnana Arasi, M. Pharm., (Ph.D.).**, Assistant Professor, Department of Pharmaceutics, who mentored me in this innovative pharmaceutical research work at every stage and took great care in making me proceed on the right path, through her valuable suggestions and inspirations.

My sincere gratitude to our beloved Principal **Dr. T.K.Ravi, M.Pharm., Ph.D., FAGE.,** for supporting and providing every need from time to time to complete this work successfully.

I express my deep sense of gratitude and indebtedness to **Dr. M. Gopal Rao, M.Pharm., Ph.D.,** Vice principal and Head, Department of Pharmaceutics, for his valuable suggestions and support during this project.

I submit my sincere thanks to our beloved Managing Trustee **Thiru. R. Vijayakumhar, Managing Trustee, M/s. SNR Sons Charitable Trust, Coimbatore** for providing all the facilities to carry out this work.

I owe my profound gratitude to **Dr. M. Gandhimathi, M.Pharm., Ph.D.,** for helping me to carry out the analytical studies and also for being a beacon of wisdom in resolving any challenges I faced during the course of work.

My sincere thanks to my dear teachers **Dr. S. Krishnan M.Pharm., Ph.D., Dr. K. Muthuswamy, M.Pharm., Ph.D., Dr. J. Bhagyalakshmi, M. Pharm., Ph.D., Dr. A.S. Manjuladevi M.Pharm., Ph.D., and Dr. R.M. Akila M.Pharm., Ph.D.** for their moral support and guidance during the course of work.

I would also like to thank **Dr. Venkataswamy, M.Sc., Ph.D., Mr.S. Muruganandham, Mrs. Dhanalaxmi** and Mrs. **Kalaivani** for their kind co-operation during this work.

I remain greatly indebted to my parents **Mr. Sunny Varkey and Mrs. Merly Sunny** and my sister **Ms. Eline Maria Sunny** for their precious love, affection and moral support which kept me on high spirits and are also the backbone for all successful endeavors in my life.

Words cannot express my gratitude to my friends **Chinju, Rahul, Sneha, Jeny, Gopinath, Haritha, Raja, Dona, Adithya, Siva, Waseem, Ansu and Sai** for their support, cooperation and inspiration during the course of my work.

I wish to extend my sincere thanks to all my batchmates, seniors and juniors who directly or indirectly helped me during my work.

I express my gratitude to all my dear teachers, friends and relatives for their support during my work.

I wish to thank **Star Colour Park** for framing project work in a beautiful manner.

Ann Maria Sunny

CONTENTS

ABBREVIATIONS

FT-IR - Fourier Transform Infrared

UV - UltraViolet

SEM - Scanning Electron Microscopy

EC - Ethyl Cellulose

PVA - Poly Vinyl Alcohol

NDDS - Novel Drug Delivery System

CD - Cyclodextrin

rpm - rotation per minute

BCS - Biopharmaceutical Classification System

cm - centimetre

et al. - and others

g - gram(s)

hrs - hour(s)

min(s) - minutes

mg - milligrams

ml - millilitre

nm - nanometer

µg - micrograms

λ_{max} - absorption maxima

mV - millivolt

KBr	-	Potassium Bromide
PDI	-	Poly Dispersity Index
r^2	-	Regression Value
vs.	-	Versus
NS	-	Nanosponges

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INTRODUCTION

The pharmaceutical and health care industry has been creating and using nano-scale materials for resolving many physical, biological and chemical problems related with the treatment of disease. The hydrophobic nature of most of the drugs presents a challenge for effective *in vivo* delivery. Shrinking materials to nano size has profoundly enhanced the efficacy of such drugs. A number of polymers have been studied and used for formulating Novel drug delivery systems (NDDS) [1].

An ideal drug therapy attains effective drug concentration at the target site for a specified period of time and minimizes general and local side effects. To obtain a desirable therapeutic response, the correct amount of drug should be transported and delivered to the site of action with subsequent control of drug input rate. The distribution of drug to other tissues therefore seems unnecessary, wasteful and a potential cause of toxicity. Targeted drug delivery is the delivery of drug to receptor, organ or any part of the body to which one wishes to deliver the drug exclusively. Targeting drug delivery has long been a problem for medical researchers i.e., how to get them to the right place in the body and how to control the release of the drug to prevent overdoses. The development of new and complex molecule called Nanosponges has the potential to solve this problem [2].

Nanosponges are made of microscopic particles with few nanometers wide cavities, in which a large variety of substances can be encapsulated. These particles possess the ability to carry both lipophilic and hydrophilic substances and thereby improving the solubility of poorly water soluble molecules. The studies conducted in this field proves that the tiny mesh-like structures called nanosponges may revolutionise the treatment of many diseases and early trials suggest this technology is up to five times more effective at delivering drugs for breast cancer than conventional methods [3].

The nanosponge is about the size of a virus with a ‘backbone’ (a scaffold structure) of naturally degradable polyester. They ‘cross link’ segments of the polyester to form a spherical shape that has many pockets (or cavities) where

drugs can be encapsulated. The polyester is biodegradable, which means that when it breaks down in the body, the drug can be released on a known schedule.

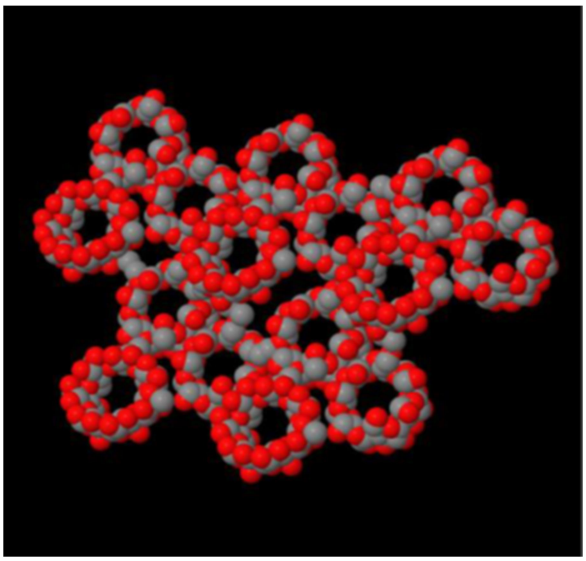


Figure 1: Cyclodextrin based nanosponges

The nanosponges are encapsulating type of nanoparticles which encapsulates the drug molecules within its core. Based on the method of associating with drugs, the nanoparticles are classified into encapsulating nanoparticles, conjugating nanoparticles and complexing nanoparticles. The encapsulating nanoparticle is represented by nanosponges and nanocapsules. Nanosponges such as alginate nanosponge, which are sponge like nanoparticles contains many holes that carry the drug molecules. The second category is conjugating nanoparticle, which links to drugs through covalent bonds. The third type is complexing nanoparticle, which attracts the molecules by electrostatic charges [4].

The nanosponges are solid in nature and can be formulated as oral, parenteral, topical or inhalational dosage forms. For oral administration, these may be dispersed in a matrix of excipients, diluents, lubricants and anticaking agents which is suitable for the preparation of tablets or capsules. For parenteral administration, these can be simply mixed with sterile water, saline or other aqueous solutions. For topical administration, they can be effectively incorporated into topical hydrogel [5].

When compared to the other nanoparticles, they are insoluble both in water and organic solvents, porous, non-toxic and stable at high temperatures up to 300°C. They are capable of capturing, transporting and selectively releasing a huge variety of substances because of their specific 3D structure containing cavities of nanometric size and tunable polarity. Furthermore, nanosponges show a notable advantage in comparison with the common nanoparticles that is, they can be easily regenerated by different treatments, such as washing with eco-compatible solvents, mild heating, stripping with moderately inert hot gases or changing ionic strength or pH [6].

The simple chemistry of polymers and cross linkers poses no problems in the preparation and this technology can be easily ramped up to commercial production levels. They can be mixed with water and used as a transport fluid. They are also used to mask unpleasant flavours, to convert liquid substances to solids. The chemical linkers allow the nanosponges to bind preferentially to the target site [7].

The nanosponges could be either in crystalline or in paracrystalline form. The loading capacity of nanosponges depends mainly on the degree of crystallisation. Paracrystalline nanosponges show different loading capacities. The nanosponges can be formulated to be of specific size and to release drugs over time by varying the proportion of cross linker to polymer. These nanosponges can be magnetized when they are synthesised in the presence of compounds having magnetic properties. The tiny shape of nanosponges enables the pulmonary and venous delivery of drug in a controlled manner [8].

Targeting Sites by Nanosponges

"Tagging" drug-loaded nanosponges ensures desired pharmacological response by targeting only disease affected cells and leaving the healthy ones unharmed. Drugs encapsulated within the nanosponge pores are shielded from premature destruction and stability of drug is enhanced. This tiny sponge circulates around the tumour cell until they encounter the surface to release their drug cargo in a sustained manner [9]. Nanosponge is three to five times more effective at decreasing tumour growth than direct injection. The targeted delivery systems of nanosponge have several basic advantages like, the drug is released at

the tumour instead of circulating widely through the body, and it is more effective for a given dosage. The nanosponges have basic features such as fewer harmful side effects as smaller amounts of the drug will come into contact with healthy tissue [5].

Difference between Nanoparticles and Nanosponges

The thin line of distinction among nanoparticles and nanosponges is the difference in porosity and size. Nanoparticles have size in nanometer whereas nanosponges have pores in nanometers while their overall size can extend up to micrometers, and are usually smaller than 5µm. Many times nanosponges have been reported as nanoporous nanoparticles / microparticles. Nanosponges show diverse domains in their structure, since they have both hydrophobic and hydrophilic groups.

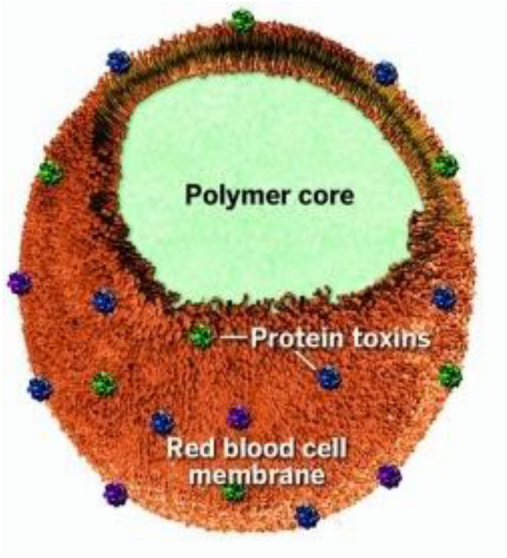


Figure 2: Polymer based nanosponges

ADVANTAGES OF NANOSPONGES

1) Being amphiphilic in nature, nanosponges can carry both hydrophobic molecules in the hydrophobic cavity and hydrophilic molecules in the spaces between the hydrophobic moieties simultaneously. Hydrophobic drugs can be loaded into the nanosponge structure to consequently increase their solubility.

2) The superior properties of nanosponges have been attributed to 'tunability', that is the ability to control the structure of particles and control the nature and size of aperture.
3) Nanosponges have the ability to produce predictable/controlled drug release [1].
4) Nanosponges can be tagged with specific linkers to target diseased cells hence achieving greater efficacy while reducing side-effects, decreasing dose and dosing frequency and in turn increasing patient compliance.
5) Nanosponges can significantly reduce the irritation of drugs without reducing their efficacy.
6) Biodegradable in nature and easy scale up for commercial production [10].
7) They mix with water and are used as a transport fluid. They can be used to mask unpleasant flavours [11].

DISADVANTAGE

The only disadvantage of this nanosponges is their ability to include only small molecules.

CHARACTERISTIC FEATURES OF NANOSPONGES [12]

- Nanosponges of specific size can be synthesized by changing the crosslinker to polymer ratio.
- They are nontoxic, porous particles, insoluble in most organic solvents and stable up to 300ºC. They are stable at the pH range of 1-11.
- They form clear and opalescent suspension in water.
- They can be reproduced by simple thermal desorption, extraction with solvents, by using microwaves and ultrasounds.
- Their three-dimensional structure allows capture, transportation and selective release of a variety of substances.
- Chemical linkers permit nanosponges to bind preferably to the target site.
- By complexing with different drugs nanosponges can form inclusion and non-inclusion complexes.
- By adding magnetic particles into the reaction mixture, magnetic properties can also be imparted to nanosponges.

POLYMERS USED IN NANOSPONGES PREPARATION [5]

There are various polymers and cross linkers are used in the preparation of nanosponges.

Polymers: Hyper cross linked Polystyrenes, Cyclodextrines and its derivatives like Alkyloxycarbonyl Cyclodextrins, Methyl β-Cyclodextrin, Hydroxy Propyl β-Cyclodextrins.

Copolymers: Poly(valerolactoneallylvalerolactone), Poly(valerolactoneallylvalero -lactone oxepanedione), Ethyl Cellulose, Poly vinyl alcohol.

Cross linker: Carbonyl diimidazoles, Carboxylic acid dianhydrides, Diarylcarbonates, Dichloromethane, Diisocyanates, Diphenyl Carbonate, Epichloridine, Gluteraldehyde, Pyromellitic anhydride, 2,2-bis (acrylamido)Acetic acid.

PREPARATION OF NANOSPONGES

Nanosponges are prepared mainly on the criteria of delivery system, polymer and nature of drug and solvents.

1) **Nanosponges prepared from hyper-cross linked β-cyclodextrins:**

β-cyclodextrin nanosponges were prepared by placing 100ml of dimethyl formamide (DMF) in a round bottomed flask and 17.42g of anhydrous β-CD was added and shaken to achieve complete dissolution. Then 9.96g of carbonyl di-imidazole (61.42m mol) was added and the solution was allowed to react for 4hrs at 1000°C. Once condensation polymerization was complete, the block of hyper cross linked cyclodextrin was roughly ground and an excess of deionised water was added to remove DMF. Finally residual by-products or unreacted reagents were completely removed by soxhlet extraction with ethanol [8].

The white powder thus obtained was dried overnight in an oven at 600°C. The fine powder obtained was dispersed in water. The colloidal part that remained suspended in water was recovered and lyophilized. The obtained nanosponges are sub-micron in dimension and with a spherical shape [7].

2) **Emulsion solvent diffusion method:**

Nanosponges can be prepared by using different proportion of ethyl cellulose and polyvinyl alcohol. The dispersed phase containing ethyl cellulose and drug

was dissolved in 20ml dichloromethane and slowly added to a definite amount of polyvinyl alcohol in 150ml of aqueous continuous phase. The reaction mixture was stirred at 1000 rpm for 2hrs in a magnetic stirrer. The nanosponges formed were collected by filtration and dried in oven at 40°C for 24hrs. The dried nanosponges were stored in vaccum desiccators to ensure the removal of residual solvents [2].

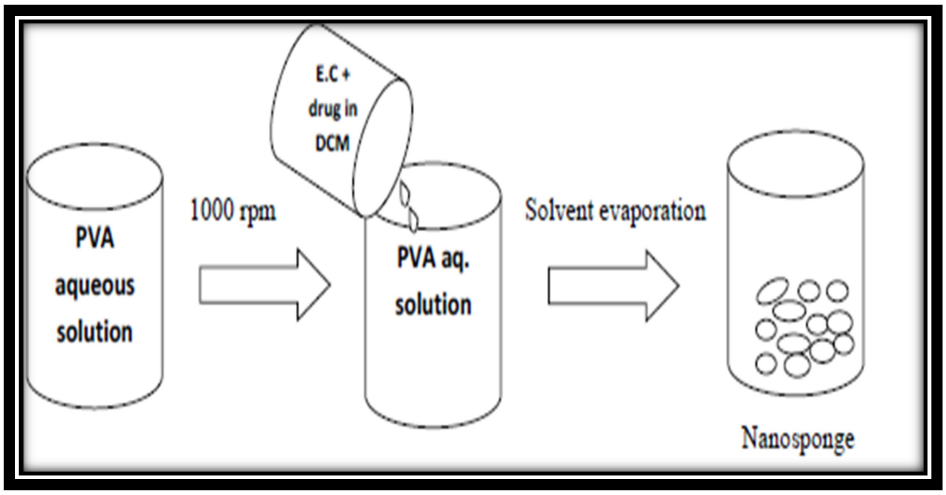


Figure 3: Preparation of nanosponges by emulsion solvent diffusion method

3) Quasi-emulsion solvent diffusion:

The inner phase is prepared using eudragit RS 100 and added to a suitable solvent. Drug to be incorporated is made into a solution and dissolved under ultrasonication at 35ºC. This inner phase added into external phase containing polyvinyl alcohol which acts as emulsifying agent. The mixture is then stirred at 1000-2000 rpm for 3hr at room temperature and dried in an hot air oven at 40ºC for 12hr [7].

4) Ultrasound- Assisted Synthesis:

In this method, polymers react with cross- linkers in absence of solvent and under sonication. Here, the polymer and cross- linker are mixed in a flask. Place the flasks in an ultrasound bath filled with water and heated to 90°C and then sonicate for 5 hrs. It was then allowed to cool and washed with water to remove the unreacted polymer. Dry the product under vacuum and store at 250°C [8].

LOADING OF DRUG INTO NANOSPONGES

Suspend the prepared nanosponges in water and sonicate to avoid the presence of aggregates and then centrifuge the suspension to collect the colloidal fraction. Separate the supernatant and then dry the sample by freeze drying. The aqueous suspension of nanosponges was prepared and dispersed the amount of the drug to be loaded in it. Maintain the suspension under constant stirring for specific time required for complexation. After complexation, separate the uncomplexed (undissolved) drug from complexed drug by centrifugation. Then obtain the solid crystals of nanosponges by solvent evaporation or by freeze drying [7].

FACTORS AFFECTING DRUG RELEASE FROM NANOSPONGES

1. Physical and chemical properties of entrapped active pharmaceutical ingredients.
2. Physical properties of sponge system such as pore diameter, pore volume, and resiliency.
3. Properties of vehicle, in which the sponges are finally dispersed.
4. Particle size, pore characteristics, and compositions can be considered as vital parameters
5. External triggers such as pressure, temperature, and solubility of actives
6. Temperature: Some entrapped actives can be too viscous at room temperature to flow spontaneously from sponges onto the skin but increased skin or environment temperature can result in increased flow rate and ultimately drug release [13].

CHARACTERIZATION OF NANOSPONGES

1. Particle size determination

The sizes of particles are maintained during polymerization for the formation of free-following powders having fine aesthetic appearance. Particle size analysis of loaded and unloaded nanosponges can be carried out by laser light diffractometry or Malvern zeta sizer [14].

2. Determination of loading efficiency

The loading efficiency of prepared nanosponge is determined by subtracting the un-entrapped drug from the total amount of drug. The un-entrapped drug must be estimated by any suitable method of analysis. The method used for separation of un-entrapped drug by gel filtration, dialysis and ultra centrifugation. The loading efficiency is calculated as:

$$Loading\ efficiency\ = \frac{Actual\ drug\ content\ in\ nanosponge}{Theoretical\ drug\ content}\ \times 100$$

3. Compatibility Studies

The drug should be compatible with the polymers which are used for the preparation of nanosponges. The compatibility of drug with adjuvants can be determined by Thin Layer Chromatography (TLC) and Fourier Transform Infra-red Spectroscopy (FT-IR). Crystalline characteristics can be studied by powder X-ray diffraction (XRD) and Differential Scanning Colorimetry (DSC) [13]

4. Zeta Potential

Zeta potential is a measure of surface charge. The surface charge of nanosponge can be determined by using Zeta sizer [15].

5. Solubility studies

The most widely used approach to study inclusion complexation is the phase solubility method described by Higuchi and Connors, which examines the effect of a nanosponge, on the solubility of drug. Phase solubility diagrams indicate the degree of complexation [16].

6. Drug release kinetics

To investigate the mechanism of drug release from the nanosponge the release data was analysed using Zero order, First order, Higuchi, Korsemeyer-Peppas models. The data can be analysed using DD solver software. The software estimates the parameters of a non-linear function that provides the closest fit between experimental observations and non- linear function [17].

7. *In vitro* release studies

In vitro release kinetics experiments are carried out using a multi compartment rotating cell. An aqueous dispersion of nanosponges (1ml) containing the drug is placed in the donor compartment, while the receptor

compartment separated by a hydrophilic dialysis membrane is filled with phosphate buffer of requires pH. The experiment is carried out for 24hr. At fixed time intervals, the receptor buffer is completely withdrawn and replaced with fresh buffer. The amount of drug in the medium is determined by the suitable analytical method and drug release is calculated to determine the release pattern [11].

8. Microscopy studies

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) can be used to study the microscopic aspects of the nanosponges. The difference in crystallization state of the raw materials and the product seen under electron microscope indicates the formation of the inclusion complexes [8].

MARKETED FORMULATIONS

Table 1: Marketed formulations of nanosponges

Drug	Administration Route	Trade Name	Dosage Form
Dexamethasone	Dermal	Glymesason	Tablet
Iodine	Topical	Mena- gargle	Solution
Alprostadil	I.V	Prostavastin	Injection
Piroxicam	Oral	Brexin	Capsule

APPLICATIONS

Nanosponges have many applications in the pharmaceutical field due to their biocompatibility and versatility. Some of them are as follows.

a) Nanosponges in Solubility Enhancement:

Presence of crosslinking agent and cavities in the nanosponge structure helps interaction with active molecules. These features allow several substances to be included and get solubilized in the thus formed cavities. The hydrophilic hydroxyl groups on the external surface remain exposed to the environment, while the hydrophobic functionality of the complex hides in the interior cavity of the cyclodextrin the net effect is that a water soluble complex is formed [18].

b) Nanosponges in Drug Delivery:

Nanosponges have spherical shape and nanometric in size making them ideal in preparing various dosage forms like topical, parenteral, aerosol, tablets and capsules. It is found that highest solubility and *in vitro* drug release is observed in inclusion complex [19].

c) Nanosponges for Protein Delivery:

The major obstacle in protein formulation development is the maintenance of the native protein structure both during the formulation process and upon the long term storage. The nanosponges were found to be stable at 300°C and high protein complexation capacity was also observed.

d) Nanosponges in Enzyme Immobilization:

The enzyme immobilization is particularly relevant for lipases, as it improves their stability and modifies properties like enantioselectivity as well as the reaction rates. As a consequence, the demand for new solid supports, suitable for family of enzymes is constantly growing [3].

e) Nanosponges as a Carrier for Delivery of Gases:

Hypoxia (deficiency of adequate oxygen supply) is related to various pathologies, from inflammation to cancer. Sometimes it can be difficult to deliver oxygen in appropriate form and doses in clinical practice. Nanosponge formulations were developed as oxygen delivery systems for topical application which were having the ability to store and to release oxygen slowly over time [20].

f) Nanosponges as Protective Agent -against Photo Degradation:

Nanosponges were prepared by encapsulating gamma-oryzanol showing a good protection from photodegradation. Gamma-oryzanol (a ferulic acid ester mixture), an anti-oxidant and usually employed to stabilize food and pharmaceutical raw materials, moreover, used as a sunscreen in the cosmetics industry. Its applications are limited due to its high instability and photodegradation. With the gammaoryzanol loaded nanosponges a gel and an O/W emulsion are formulated [7].

g) Modulating Drug Release:

A drug loaded into the nanosponge is retained and released slowly over time. Hydrophilic nanosponges are employed to enhance the drug absorption

across biological barriers, to modify the drug release rate and as a potent drug carrier in immediate release formulations. Hydrophobic nanosponges are utilized as sustained release carriers for water soluble drugs, including peptide and protein drugs and they protect the drug during its passage through the stomach. This drug is released very slowly at pH 1.1, whereas release is faster if pH is raised to 7.4.

h) Effective Delivery Carriers:

Antitumor drugs such as paclitaxel, camptothecin and tamoxifen shows bioavailability problem (because of poor aqueous solubility) hence nanosponges can be used as vehicles in order to improve their solubility as well as bioavailability. Complexes showed high effect than that of the drug alone. After loading the drug in nanosponges the mean absolute bioavailability of paclitaxel was increased and found to be 2.5 fold higher than the plain drug [21].

Future Prospects

Nanosponges are effective carriers for targeted delivery of drugs to lungs, liver and spleen. A simple approach for formulating Palladium/Silver and Palladium/Silver/Aluminium nanosponges, which contain network of nanowires has been reported. This strategy establishes the first time preparation of alloy nanosponges with network nanowires via self-regulated reduction of sodium dodecylsulfate (SDS) and adding the second or third metal salt during synthesis without additional reducing agent. Further studies on kinetics and biochemical interactions of nanosponges within organisms are vital. These studies must mainly focus on research on nanosponges translocation pathways, its accumulation in the targeted area, short and long-term toxicity, their interactions with cells, the receptors and signalling pathways involved, cytotoxicity, and their surface functionalization for an effective phagocytosis [22]. There is only a sparse knowledge about the effects of nanosponges exposure on the lymphatic and immune systems, as well as various organs. In order to clarify the possible role of nanosponges in diseases recently associated with them (such as Crohn's disease, neurodegenerative diseases, autoimmune diseases, and cancer), nanoscale characterization techniques should be used to a larger extent to identify nanosponges at disease sites in affected organs or tissues, and to establish relevant interaction mechanisms. The cytotoxicity of the nanoparticles or their degradation

product remains a major problem and also the improvement in the biocompatibility obviously is a serious concern for the future [7].

SILYMARIN

Silymarin, a potential phytochemical compound obtained from the seeds of *Silybum marianum* (milk thistle) plant has been used as a hepatoprotective agent for more than a decade. Silymarin shows strong anti-oxidant effect through scavenging free radicals and inhibiting peroxidation of lipids. The drug is well tolerated and has relatively few adverse effects. The effectiveness of silymarin as hepatoprotective agent was diminished by its poor water solubility and low bioavailability. The poor bioavailability is mainly due to extensive metabolism, poor aqueous solubility and rapid excretion through urine and bile, and low permeability across intestinal epithelial cells. Besides silymarin has been extensively studied *in vitro* and *in vivo* for its cancer chemopreventive potential against various cancers.

The oral bioavailability of silibinin, however, is extremely low (b1%) caused primarily by its poor gut absorption and phase II metabolism in the liver. It was determined that gut permeability was not the rate-limiting step in the gut absorption of silibinin; instead it was the slow dissolution rate of silibinin due to its poor solubility in the gastrointestinal fluid. The high incidence of administration of silymarin together with its short half-life and poor bioavailability proposed great scope for the proposal and development of nanoparticulate drug delivery systems [23].

Silymarin as Anticancer Agents

Several studies have demonstrated Silymarin's anticancer effects by causing cell arrest and inducing apoptosis in different types of cancers. Silymarin induces apoptotic cell death in CH11-treated human malignant melanoma A375-S2 cells through an increased expression of Fas-associated proteins with death domain (FADD), which is a downstream molecule of the death receptor pathway, subsequent to the cleavage of procaspase-8 that induces apoptosis [24].

Breast cancer is the leading cause of cancer-related deaths among women in the world, and the metastasis of the cancerous cells is responsible for over 90%

of the deaths of these cancer patients. The nano sized drug delivery system (NDDS) is a promising strategy for increasing the accumulation of drugs in the tumour because of its enhanced penetration and retention (EPR) effects and for minimizing side effects [25].

Although the exact mechanisms involved in antineoplastic effects of silymarin in breast cancer have not been identified, possible underlying explanations include induction of G1 arrest and apoptosis through inhibiting cyclin- dependent kinases activity and epidermal growth factor receptor signalling, and increasing Cip1/p21 and p27.Breast cancer is a major health problem more commonly seen in the developed countries [26].

Silymarin Nanoformulations

Although considerable recent approaches have been made through nanotechnology by employing self-nano emulsifying drug delivery system, PLGA, chitosan, stearic acid modified polysaccharide and polymeric nanoparticles, the bioavailability problems of silymarin have not been solved to date [23].

Hence, not coincidentally, a majority of studies on bioavailability enhancement of silibinin set their aims at improving the dissolution rate by means of nanonization to take advantage of the large specific surface areas afforded by nanoparticles. Various nanoformulation platforms ranging from liposomes, solid lipid nanoparticles, and polymer nanoparticles to porous silica nanoparticles and nano-emulsions have been employed as delivery vehicles for silibinin. These nanoformulation strategies, however, possess a major drawback in their low silibinin payloads. The low payload leads to a high dosing requirement to achieve the therapeutic effect in which a large fraction of the administered dose is made up of carrier materials that not only end up wasted, but also possibly have adverse health effects due to their large amount. Moreover, the high dosing requirement would make therapy regimen of silibinin too costly for most patients, hence limiting its potential for widespread clinical applications [27].

OPTIMIZATION:

Optimization is the process of finding out the best way of using existing resources while taking into account of the factors that influences decision in any experiment.

Advantages of optimization:

- Reduce the cost
- Save the time
- Reduce chances of error
- Reproducibility, innovation and efficiency

The objectives of the present work were to improve the solubility and thereby the bioavailability of silymarin by formulating it into nanosponges. Subsequently the prepared silymarin nanosponges were investigated for their particle size, stability, preparation efficiency (silymarin utilisation, overall yield) and drug release.

RATIONALE FOR THE STUDY

Criteria for Drug Selection:

- Molecular weight of drug must be between 100-400D.
- Structure should contain no more than 5 condensed rings.
- Solubility of drug must be less than 10mg/mL.
- Melting point must be less than 250°C.

WHY SILYMARIN ?

- Silymarin even though has no lipophilic structure in its molecule has low aqueous solubility.
- Under BCS, silymarin is classified as class II drug i.e poorly soluble and highly permeable drug.
- Silymarin was found to possess poor absorption, rapid metabolism and ultimately poor oral bioavailability.
- Only 20-50 % of silymarin is being absorbed after oral administration from the GIT after which it undergoes entero-hepatic circulation.[28]
- So the drug release patterns of silymarin have to be augmented by means of various solubility enhancement techniques like nanonization.
- Number of studies has established the cancer chemopreventive role of silymarin in both *in vivo* and *in vitro* models.
- Several studies have demonstrated silymarin's anticancer effects by causing cell cycle arrest and inducing apoptosis in different type of cancers like breast cancer, lung cancer, colorectal cancer, oesophageal cancer prostate cancer etc. [29].

LITERATURE RIVIEW

- **Priyanka *et al.* (2018)** formulated ibuprofen loaded nanosponges for topical application. Emulsion solvent diffusion method was selected to prepare ibuprofen loaded nanosponges using different ratios of drug: polymer. The obtained nanosponges have been evaluated for physicochemical characteristics and *in vitro* release studies. The shape and morphology of drug loaded nanosponges were investigated and confirmed by SEM. FTIR results were in agreement with standard spectral studies and moreover it was identified that there was no interaction between drug and polymer. Entrapment efficiency of the NS was found to be around 70.41%. The production yield and *in vitro* release studies was also good. Overall this study resulted in porous nature of nanosponges which provides a channel for the release of the drug and the method is quick and reproducible [30].

- **Sornsuvit *et al.* (2018)** aimed to determine the pharmacokinetic parameters and bioavailability of silymarin 140mg self micro-emulsifying drug delivery system (SMEDDS) formulation. An open-label, single-dose pharmacokinetic study was conducted. Twelve healthy volunteers were included in the study. After the volunteers had fasted overnight for 10 h, a single-dose generic silymarin 140mg SMEDDS soft capsule was administered. Then 10ml blood samples were taken at 0.0, 0.25, 0.50, 0.75, 1.0, 1.33, 1.67, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0, and 12.0 h. The plasma silymarin concentrations were analysed using validated LC-MS/MS. The pharmacokinetic parameters were analysed and calculated. The pharmacokinetic parameters were calculated after silymarin had been administered as a single capsule. The mean (range) C_{max} was 812.43 (259.47–1505.47) ng/ml at 0.80 (0.25–1.67) h (t_{max}). The mean K_e and $t_{1/2}$ were 0.5386 h^{-1} and 1.91 h respectively. The silymarin SMEDDS formulation soft capsule showed rapid absorption and high oral bioavailability [31].

- **Gangadharappa *et al.* (2017)** proposed the study to improve the solubility of Celecoxib using β-cyclodextrin and NN-methylene bisacrylamide nanosponge hydrogel formulation. Celecoxib is an extremely lipophilic and poorly water soluble NSAID which has gastrointestinal side effects when used chronically.

Solubility of celcoxib is 7.6µg/ml and therefore the oral bioavailability of celecoxib is 40% when administered as a capsule dosage form. Nanosponges of celecoxib were prepared and characterized by differential scanning colorimetry, X-ray diffractometry, FT-IR analysis and evaluated by Zeta-potential and Polydispersity index and drug entrapment efficiency. Further, celecoxib nanosponges were dispersed in 1% carbopol 934 hydrogel and the gel was evaluated for its viscosity, pH, spreadability, *in vitro* diffusion. *In vivo*, pharmacokinetic and skin irritation studies conducted using rats. Solubility of freeze dried nanosponge particles were in the range of 230 - 490 µg/ml, which indicates 30 to 65 fold increases in the solubility compared to pure drug. These results confirm that nanosponge formulation is ideal for increasing the solubility and bioavailability of poorly water soluble drug like celecoxib [32].

- **Shilpa *et al.* (2017)** developed and characterized silybin loaded solid lipid nanoparticle gel for irritant contact dermatitis (ICD). sICD is associated with reduced skin water content, emerging in dry skin condition and relapsing eczema. In this study, the silybin loaded nanoparticles was prepared by the ultrasonic probe sonication method and further evaluated for particle size and entrapment efficiency. Results of optimized batch showed mean particle size 139±0.35 nm and entrapment efficiency 90.97±0.91%. Optimized batch was freeze dried and characterized by field emission scanning electron microscopy (FE-SEM), it shows particles are in nano range, with spherical morphology and smooth surface. Finally, the nanoparticles were incorporated into gel for convenient topical application. The gel were evaluated for *in vitro* skin occlusivity, skin irritation and *ex vivo* diffusion and deposition study and further compared with plain gel. Efficacy of gel on dinitrochlorobenzene (DNCB) induced ICD mice were evaluated by skin water content, ear swelling and histopathology. *Ex vivo* study of gel exhibited prolonged drug release, whereas the skin irritation study shows no irritancy [33].
- **Cristian Radu *et al.* (2017)** aimed to address one of the major challenges of the actual era of nanomedicine namely, the bioavailability of poorly water soluble drugs such as Silymarin by developing new, biodegradable, and biocompatible nanosized shuttles for Silymarin targeted delivery in colon-cancer

cells. The design of these 100 nm sized carrier nanoparticles was based on natural polymers and their biological properties such as cellular uptake potential, cytotoxicity and 3D penetrability were tested using a colon cancer cell line (HT-29) as the *in vitro* culture model. Comparative scanning electron microscopy (SEM) and atomic force microscopy (AFM) measurements demonstrated that the Silymarin loaded Poly(3-HydroxyButyrate-co-3-HydroxyValerate) (PHBHV) nano carriers significantly decreased HT-29 cells viability after 6 and 24 h of treatment. Moreover, *in vivo* like toxicity studies on multicellular tumour spheroids showed that the Silymarin loaded PHBHV nano carriers are able to penetrate 3D micro tumours and significantly reduce their size [34].

- **El-Nahas *et al.* (2017)** formulated and characterized silymarin-loaded Eudragit nanoparticles (SNPs) and evaluated their hepatoprotective and cytotoxic effects after oral administration. SNPs were prepared by nanoprecipitation technique and were evaluated for particle size, entrapment efficiency, TEM, solid-state characterization, and *in vitro* drug release. The hepatoprotective activity was evaluated after oral administration of selected SNPs in carbon tetrachloride-intoxicated rats. Potential *in vivo* acute cytotoxicity study was also assessed. Morphology of the selected SNPs revealed spherical and uniformly distributed nanoparticles. DSC and FT-IR studies suggested the presence of silymarin in an amorphous state and absence of chemical interaction. The hepatoprotective evaluation of the selected SNPs in CCl_4 intoxicated rats revealed significant improvement in the activities of different biochemical parameters compared to the marketed product. The histopathological and toxicity studies were also carried out. The obtained results suggested that the selected SNPs were safe and potentially offered enhancement in the pharmacological hepatoprotective properties of silymarin [35].

- **Ahmad *et al*. (2017)** developed and optimized nanoemulsion of silymarin. Nanoemulsion was made by aqueous titration method. Sefsol 218, Kolliphor RH40 and polyethylene glycol 400 were used as oil phase, surfactant and co-surfactant while distilled water acted as an aqueous phase. Nanoemulsion was characterized on the basis of particle size, viscosity, electrical conductivity and refractive index. Further, *in vitro* release, *in vivo* pharmacokinetic study, stability

study and cancer cell line studies were also performed. The stability study also showed considerably stable formulations at refrigerator temperature as compared with room temperature. The cancer cell line studies also confirmed that silymarin nanoemulsion reduced the cell viability. The results concluded that nanoemulsion may be an efficient carrier for oral delivery of silymarin against human hepatocellular carcinoma without damaging normal cells [36].

- **Hsuan *et al*. (2017)** assembled flavonoid silymarin and zein into spherical silymarin-zein nanoparticles that could be effectively adsorbed onto bacterial cellulose nanofibers. Silymarin-zein nanoparticles greatly changed the wettability and swelling property of bacterial cellulose films due to the formation of nanoparticles/nanofibers nanocomposites. Silymarin-zein nanoparticles enhanced the release of sparingly soluble silymarin from the nanocomposite films. The active films showed more effective antioxidant and antibacterial activities as compared with pure films and thus were able to protect salmon muscle from deterioration and lipid oxidation. These findings suggest that the nanoparticle/nanofiber composites may offer a suitable platform for modification of bacterial cellulose films with improved drug release properties and biological activities [37].
- **Younis *et al.* (2016)** developed nano-formulations of silymarin (SM), a drug commonly used for liver diseases, to overcome its poor solubility and poor bioavailability; antifibrotic effect of these formulations has not been tested yet. This study was aimed to formulate and evaluate silymarin-loaded eudragit RS100 nanoparticles (SMnps) and to test the capability of SMnps to reverse an established fibrosis model. SMnps were prepared by solvent evaporation and nano-precipitation techniques. The influence of drug: polymer ratio, concentration of surfactant in the aqueous phase on particle size, drug entrapment efficiency (EE) % and *in vitro* drug releases were investigated. Formulations of SMnps represent a step forward in the field of anti-fibrotic drug development [38].
- **Bhagyashree Subhash P. and Dr. Mohite S. K (2016)** aimed to produce controlled release artesunate nanosponges for topical and oral delivery. Nanosponges using three different polymers ethyl cellulose, Poly (methyl methacrylate) and Pluronic F-68 (poloxamer 188) were prepared successfully

using PVA as surfactant by emulsion solvent evaporation method. The effects of different drug: polymer ratios, surfactant concentration, stirring speeds and time, sonication time on the physical characteristics of the nanosponges as well as the drug entrapment efficiency of the nanosponges were investigated. Particle size analysis and surface morphology of nanosponges were performed. The scanning electron microscopy of nanosponges showed that they were spherical in shape and spongy in nature. These small sponges can circulate around the body until they encounter the target site and stick on the surface and began to release the drug in a controlled and predictable manner which is more effective for a particular given dosage [39].

- **Amiri *et al.* (2016)** prepared nanoniosomal silibinin and evaluate its cytotoxicity in the T-47D breast cancer cell line. Silibinin is a chemotherapeutic agent active against cancer. Niosomes are biodegradable, biocompatible, safe and effective carriers for drug delivery. Niosomes were prepared by reverse phase evaporation of a mixture of span 20, silibinin, PEG-2000 and cholesterol in chloroform and methanol solvent (1:2 v/v). The solvent phase was evaporated using a rotary evaporator and the remaining gel phase was hydrated in phosphate buffer saline. Mean size, size distribution and zeta potential of niosomes were measured with a Zetasizer instrument and then nanoparticles underwent scanning electron microscopy. The drug releasing pattern was evaluated by dialysis and the cytotoxicity of nanoniosomes in T-47D cells was assessed by MTT assay. The amount of encapsulated drug and the level of drug loading were determined 98.6 ± 2.7% and 22.3 ±1.8%, respectively; released drug was estimated about 18.6±2.5% after 37 hours. The cytotoxic effects of nanoniosome were significantly increased when compared with the free drug. This study finding suggests that silibinin nanoniosomes could serve as new drug formulation for breast cancer therapy [40].
- **Zhang *et al.* (2016)** carried out a study with the objective to prepare silyblin nanoparticles (NPs) and optimize the prepared nanoparticles using central composite rotatable design-response surface methodology. Hepatocellular carcinoma (HCC) is a most common liver malignancy. HCC was induced in rats by supplementing 100 mg/L of diethylnitrosamine (DENA) in drinking water for 8 weeks. Saline, silybin 30 mg/kg body weight and nanoformulation of silybin

equivalent to silybin dose were administered orally to 3 groups of 6 animals each. Anticancer activity was evaluated by counting the liver nodules. The results showed that silybin NPs under optimized conditions gave rise to the entrapment efficiency (EE) of 88%, drug loading of 15%, mean diameter of 216 nm of the NPs prepared and zeta potential value of -15 mV. In rats treated with silybin NPs, the number of neoplastic nodules was significantly lower, the animals did not exhibit decrease in mean body weight, the number of liver nodules was reduced by >93% with significantly high localization in the liver. It was concluded that orally administered silybin NPs showed improved efficacy and safety compared to silybin for the treatment of HCC in rats [41].

- **Li J. *et al.* (2016)** determined the expression status and role of AMPK in esophageal squamous cell carcinoma (ESCC) and investigated whether silibinin, a nontoxic natural product, could activate AMPK to inhibit ESCC development. Emerging evidence suggested that activation of adenosine monophosphate-activated protein kinase (AMPK) may suppress cancer growth. We found that silibinin induced apoptosis, and inhibited ESCC cell proliferation *in vitro* and tumorigenicity *in vivo* without any adverse effects. Silibinin also markedly suppressed the invasive potential of ESCC cells *in vitro* and their ability to form lung metastasis in nude miceThis preclinical study supported that AMPK is a valid therapeutic target and suggested that silibinin may be a potentially used as a therapeutic agent and chemo sensitizer for oesophageal cancer [42].

- **Viswanad V. and Jilsha G. (2015)** formulated cephalexin into nanosponge loaded hydrogel as it can enhance skin permeation. Nanosponges of cephalexin were prepared using hydroxyl ethyl cellulose and poly vinyl alcohol by emulsion solvent evaporation method. The particle size and entrapment efficiency was found in the range of 200-400 nm and 88.5%- 95.6% respectively. Based on the characterization, nanosponges with high entrapment efficiency and least particle size were selected for hydrogel formulation. Five different formulations of hydrogels were prepared by using carbopol 934 with varying concentration of penetration enhancer (propylene glycol) and various evaluation studies were carried out. The *in vitro* release studies revealed that the formulation with higher concentration of penetration enhancer (15% propylene glycol) showed greater

drug release. From the kinetic study, the best linearity was found with first order and Higuchi's equation [43].

- **Aldawsari *et al.*2015)** carried out a research aiming to formulate lemongrass-loaded ethyl cellulose nanosponges with a topical hydrogel with an enhanced antifungal effect and decreased irritation. Lemongrass-loaded ethyl cellulose nanosponges were formulated. The emulsion solvent evaporation technique was used for the preparation of the nanosponges. The nanosponge dispersions were then integrated into carbopol hydrogels (0.4%). The prepared formulations were evaluated for particle size, citral content, and *in vitro* release. Results revealed that all the nanosponge dispersions were nanosized, with satisfactory citral content and sustained release profiles. Statistical analysis revealed that both ethyl cellulose : polyvinyl alcohol ratio and stirring rate have significant effects on particle size and percentage released after 6 hours; however, the effect of the stirring rate was more prominent on both responses. The selected hydrogel formulation, F9, was subjected to surface morphological investigations, using scanning and transmission electron microscopy, where results showed that the nanosponges possess a spherical uniform shape with a spongy structure, the integrity of which was not affected by integration into the hydrogel [44].
- **Srinivas P. and Jahnavi Reddy A. (2015)** formulated controlled release Isoniazid (INH) nanosponges for topical delivery. Nanosponges using ethyl cellulose polymer were prepared successfully using PVA as surfactant by emulsion-solvent evaporation method. The effects of different surfactant concentration, drug: polymer ratio, stirring speeds, stirring time and sonication time on the physical characteristics of the nanosponges as well as the drug entrapment efficiency of the nanosponges were investigated. Particle size analysis and surface morphology of nanosponges were performed. The scanning electron microscopy of nanosponges showed that they were spherical in shape and spongy in nature. The particle size of the formulations was found to be 124 nm and the drug entrapment efficiency was found to be in the range of 47.18% to 74.86 %. The optimized Nanosponge formulation (I6) was selected for formulating nanogels using various gelling agents like Carbopol 934, Carbopol 940, HPMC K4M and studied for pH, viscosity and *in vitro* drug release. Of the various

formulations prepared, F2 was found to show the maximum sustained drug release of 74.26% in 10 hours [45].

- **Seema *et al.* (2015)** developed curcumin loaded nanosponges which increased the bioavailability and retention time at the colon. Clinical importance of curcumin in colon diseases like inflammatory bowel diseases (Chron's disease and ulcerative colitis) irritable bowel syndrome and colon cancer has been reported. Due to small size and porous nature of the novel delivery system they can easily bind to poorly-soluble drugs within their matrix and prevent drug from rapid metabolism and excretion, improve their bioavailability. The nanosponge of curcumin is prepared by solvent evaporation method. Further the formulated nanosponges are evaluated for its phase solubility, particle size determination, zeta potential, SEM, TEM analysis, loading efficiency, and production yield and *in vitro* release study [46].
- **Lockhart *et al.* (2015)** reported the synthesis and encapsulation of polyester nanosponge particles (NPs) co-loaded with tamoxifen (TAM) and quercetin (QT) to investigate the loading, release and *in vitro* metabolism of a dual drug formulation. The NPs were made in two variations, 4% and 8% crosslinking densities, to evaluate the effects on metabolism and release kinetics. The stability of the formulation was established in simulated gastrointestinal fluids, and the metabolism of TAM was shown to be reduced 2-fold and 3-fold for NP-4%s and NP-8%s, respectively, while QT metabolism was reduced 3 and 4-fold. This work demonstrates the suitability of the nanosponges not only as a dual release drug delivery system but also enabling a regulated metabolism through the capacity of the nano network. The variation in crosslinking enables a dual release with tailored release kinetics and suggests improved bioavailability aided by a reduced metabolism [47].
- **Wang Y *et al*., (2014)** aimed to identify the CXCR4 antagonists that could reduce and/or inhibit tumour metastasis from natural products. C-X-C chemokine receptor type 4 (CXCR4) signalling has been demonstrated to be involved in cancer invasion and migration; therefore, CXCR4 antagonist can serve as an anti-cancer drug by pre-venting tumor metastasis. According to the molecular docking screening, we reported here silibinin as a novelCXCR4 antagonist. Biochemical

characterization showed that silibinin blocked chemokine ligand 12(CXCL12)-induced CXCR4 internalization by competitive binding to CXCR4, therefore inhibiting down-stream intracellular signalling. The inhibition of silibinin was also observed in MCF-7/CXCR4 cells rather than MCF-7 cells that express low level of CXCR4. This work demonstrated that silibinin is a novel CXCR4 antagonist that may have potential therapeutic use for prevention of tumour metastasis [48].

- **Jana *et al.* (2014)** carried out a study to improve the solubility and bioavailability of nebivolol. The present study attempts to overcome these issues through nanoparticulate delivery system using widely used carrier EudragitRS100. The solvent evaporation (single emulsion) technique was used for developing nanoparticles. The impact of formulation and process variables on particle size and entrapment efficiency was studied to optimize the formulation. The physico-chemical characterization confirmed the particle size in nano range with smooth and spherical morphology. Further, Fourier transforms infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC) studies confirm compatibility of drug–polymer combination. The *in vitro* drug release study of the prepared nanoparticles showed prolongation of drug release with reduced burst release in comparison with pure drug powder [49].
- **Sadiq and Abdul Rassol (2014)** aimed to develop and evaluate silibinin (SIL) loaded solid lipid nanoparticles (SLN) in an attempt to increase its oral bioavailability and targeting the lower part of GI tract. Solvent emulsification-evaporation method with slight modification was used to prepare the SLNs and glyceryl monostearate (GMS), trimyristin (TM), tripalmitin (TP) and tristearin (TS) were investigated as solid lipid matrix. Tween 20 (T20), tween 80 (T80), polyvinyl alcohol (PVA), poloxamer 188 (P188), sodium cholate (SC) and sodium deoxycholate (SDC) were investigated as emulsifiers. The formulations were evaluated for entrapment efficiency (EE), particle size distribution and *in-vitro* release profile. Furthermore, the optimized formula (F2) was further investigated by TEM, FTIR and DSC studies. All the prepared SLNs are within submicronal range and acceptable polydispersity index (PI). The EE of the prepared SLNs were from (64.67±4.51%) to (87.00±2.00%). FTIR and DSC studies were done for the final formula (F2) which contains TS as solid lipid matrix and T80 and

P188 as emulsifier combination and it showed no drug – excipient incompatibility and suggests formation of an amorphous solid solution. It can be concluded that SIL could easily incorporated into SLN containing TS and P188 for oral use [50].

- **Poojaa *et al.* (2014)** tried to develop silibinin loaded chitosan nanoparticles so as to improve its bioavailability. This study presents fabrication and characterization of chitosan-tripolyphosphate based encapsulation of silibinin. Various preparations of silibinin encapsulated chitosan-tripolyphosphate nanoparticles were studied for particle size, morphology, zeta-potential, and encapsulation efficiencies. Preparations were also evaluated for cytotoxic activities *in vitro*. The optimized silibinin loaded chitosan nanoparticles were of 263.7 ± 4.1 nm in particle size with zeta potential 37.4 ± 1.57 mV. Nanoparticles showed high silibinin encapsulation efficiencies (82.94 ± 1.82%). No chemical interactions between silibinin and chitosan were observed in FTIR analysis. Powder X-ray diffraction analysis revealed transformed physical state of silibinin after encapsulation. Surface morphology and thermal behaviour were determined using TEM and DSC analysis. Encapsulated silibinin displayed increased dissolution and better cytotoxicity against human prostate cancer cells (DU145) than silibinin alone [51].
- **Ramteke *et al.* (2014)** proposed a detailed study about the various mathematical models of drug dissolution. When a new solid dosage form is developed, it is very important to study drug release or dissolution. The quantitative analysis of values obtained in dissolution or release rates is easier when mathematical formulae are used to describe the process. The mathematical modelling helps to optimize the design of a therapeutic device to yield information on the efficacy of various release models. In this paper review of the different mathematical models used to determine the kinetics of drug release from drug delivery systems such as, zero order, first order, Hixson-Crowell,Higuchi, Weibull, Korsemeyer-Peppas, Hopfenberg, Baker-Lonsdale and Gompertz model were carried out [52].
- **Raja *et al.* (2013)** formulated nanosponge loaded with ciprofloxacin antibiotic and resulted in sustained release. The drug is acid labile and hence it is entrapped with ethyl cellulose for its sustained release. As the drug is made into

nanoparticle the density was found to be increased. Among all the formulated batches starting from F1 through F5 the final batch (F5) is considered as the best entrapped (90.80%) nanosponge with greater percentage drug release (99.4%). The characterization by SEM concluded the appearance as a nanosponge [53].

- **Srinivas and Sreeja (2013)** produced controlled release Voriconazole nanosponges for topical and oral delivery. Nanosponges using three different polymers ethyl cellulose, Poly (methyl methacrylate) and Pluronic F-68 (poloxamer 188) were prepared successfully using PVA as surfactant by emulsion solvent evaporation method. The effects of different drug: polymer ratios, surfactant concentration, stirring speeds and time, sonication time on the physical characteristics of the nanosponges as well as the drug entrapment efficiency of the nanosponges were investigated. Particle size analysis and surface morphology of nanosponges were performed. The scanning electron microscopy of nanosponges showed that they were spherical in shape and spongy in nature. The particle size of the optimized formulations was in the range of 200-400nm and the drug entrapment efficiency was found to be in the range of 69.8 % to 72.5%. These nanosponge formulations were prepared as gel using carbopol 971P and studied for pH, viscosity, *in vitro* drug release, antimicrobial activity. Among the various formulations prepared E2, P2 and F2 were found to show the maximum drug release of 92.76%, 91.84% and 95.88% respectively at 1:2 drug: polymer ratio at the end of 48 hours [54].

- **Yeol Yang *et al.* (2013)** developed a novel silymarin-loaded solid nanoparticle system using Shirasu porous glass (SPG) membrane emulsification and a spray-drying technique. The physicochemical characteristics of these nanoparticles were determined by scanning electron microscopy, differential scanning calorimetry, and powder X-ray diffraction. Their dissolution, bioavailability, and hepatoprotective activity in rats were assessed by comparison with a commercially available silymarin-loaded product. Formulation of a silymarin-loaded nanoemulsion, comprising silymarin, castor oil, polyvinylpyrrolidone, Transcutol HP, Tween 80, and water at a weight ratio of 5/3/3/1.25/1.25/100 was accomplished using an SPG membrane emulsification technique at an agitator speed of 700 rpm, a feed pressure of 15 kPa, and a

continuous phase temperature of 25°C. This resulted in generation of comparatively uniform emulsion globules with a narrow size distribution. Silymarin was located in unaltered crystalline form in the nanoparticles. The drug dissolved rapidly from the nanoparticles, reaching nearly 80% within 15 minutes, indicating three-fold better dissolution than that of the commercial product [55].

- **Ying Ho *et al.* (2012)** investigated the *in vitro* and *in vivo* bioactivities of silibinin (SB), paclitaxel (PTX) and SB and PTX in combination (SB+PTX) against murine metastatic mammary 4T1 cancer cell line. Isobologram and combination index (CI) analyses showed that SB and PTX can function synergistically in the inhibition of 4T1 cell proliferation with a CI value < 1. Flow cytometry and Western blot analyses demonstrated that both drugs deregulated cell-cycle mediators and induced apoptosis in 4T1 cells. A real-time *in vivo* bioluminescence imaging system to monitor the breast cancer cell metastasis and *in vivo* study indicated that SB co-treated with PTX can significantly suppress lung metastasis of 4T1 cells likely through inhibiting cell proliferation and angiogenesis. Together, this study demonstrates that SB could act synergistically with PTX in 4T1 cells, providing a therapeutic option for highly metastatic triple negative breast cancer [56].
- **Fehér and Lengyel (2012)** studied the activity of silymarin also called as milk thistle in liver diseases and also chemopreventive effect. Liver cirrhosis, non-alcoholic fatty liver and steatohepatitis are risk factors for hepatocellular carcinoma (HCC). The silymarin exerts membrane-stabilizing and antioxidant activity, it promotes hepatocyte regeneration; furthermore it reduces the inflammatory reaction, and inhibits the fibrogenesis in the liver. These results have been established by experimental and clinical trials. According to open studies the long-term administration of silymarin significantly increased survival time of patients with alcohol induced liver cirrhosis [57].
- **Das S. *et al.* (2011)** formulated silymarin nanoparticles by nanoprecipitation in polyvinyl alcohol stabilized Eudragit RS100® polymer. Process parameter optimization provided 67.39% entrapment efficiency and a Gaussian particle distribution of average size 120.37 nm. Silymarin release from the nanoparticles was considerably sustained for all formulations. Silymarin

nanoparticles were strongly protective against hepatic damage when tested in a paracetamol overdose hepatotoxicity model. Nanoparticles recorded no animal death even when administered after an established paracetamol-induced hepatic necrosis [58].

- **Zhang Y *et al.* (2010)** gave details regarding different mathematical approaches that have been proposed to assess the similarity between two drug dissolution profiles particularly DD solver. The purposes of this article were: (1) to describe the development of a software program, called DD Solver, for facilitating the assessment of similarity between drug dissolution data; (2) to establish a model library for fitting dissolution data using a nonlinear optimization method; and (3) to provide a brief review of available approaches for comparing drug dissolution profiles. Sample runs of the program demonstrated that the results were satisfactory, and DD Solver could be served as a useful tool for dissolution data analysis [59].
- **Zhanga *et al.* (2009)** successfully prepared micronized silybin particles by emulsion solvent diffusion method. Uniform spherical and rod-shaped particles could be obtained using sodium dodecyl sulfate (SDS) concentration of 0.1 wt% at 30 and 15ºC, respectively. The characterization of silybin particles by SEM and particle size distribution (PSD) indicated that with the increase of temperature from 15 to 30ºC, the prepared particles became bigger and had a tendency to turn into spherical shapes; with the increase of SDS concentration from 0.02 - 0.1 wt%, the span of PSD became narrower while the mean particle size kept almost unchanged. XRD patterns and FT-IR spectra showed that the spherical and rod-shaped silybin particles possessed decreased crystallinity; however, the chemical structure and components were similar to those of the commercial silybin powder. Dissolution tests demonstrated that both of the spherical and rod-shaped silybin particles exhibited significantly enhanced dissolution rate when compared to the commercial silybin powder [60].
- **Snehalatha *et al.* (2008)** prepared etoposide-loaded nanoparticles using nanoprecipitation and emulsion solvent evaporation techniques using polylactide-co-glycolic acid and poly(ε-caprolactone) in presence of Pluronic F68, respectively. Effect of formulation variables like stabilizer concentration, amount

of polymer, and drug was studied. The methods produced nanoparticles with good entrapment efficiency of around 80%. Recovery of nanoparticles was as high as 95% and drug content was around 1.5%. Increase in lactide content decreased the release of etoposide *in vitro* and poly(ε- caprolactone) nanoparticles retarded etoposide release for 48 hr [61].

- **Mahmoud *et al.* (2006)** formulated silymarin hybrid liposomes for buccal administration after investigating their stability and *in vivo* hepatoprotective efficiency. Silymarin loaded hybrid liposomes composed of lecithin (L), cholesterol(Ch), stearyl amine (SA) and Tween 20 (T20) in molar ratio of (9:1:1:0.5) were prepared. Their stability upon storage was studied at 4 ◦C and at ambient conditions. Stored samples were analysed for percent encapsulation, drug release, particle size, turbidity measurement and visual changes. Characterization of the blend between phospholipid and silymarin was done using FT-IR and DSC which indicated a possible interaction. The stabilized formula of silymarin hybrid liposomes was evaluated upon buccal administration regarding its hepatoprotective activity against carbon tetrachloride-induced oxidative stress in albino rats. The introduced silymarin hybrid liposomes produced a significant decrease in both transaminase levels when challenged with CCl_4 (intraperitonially) in comparison with orally administered silymarin suspension [62].

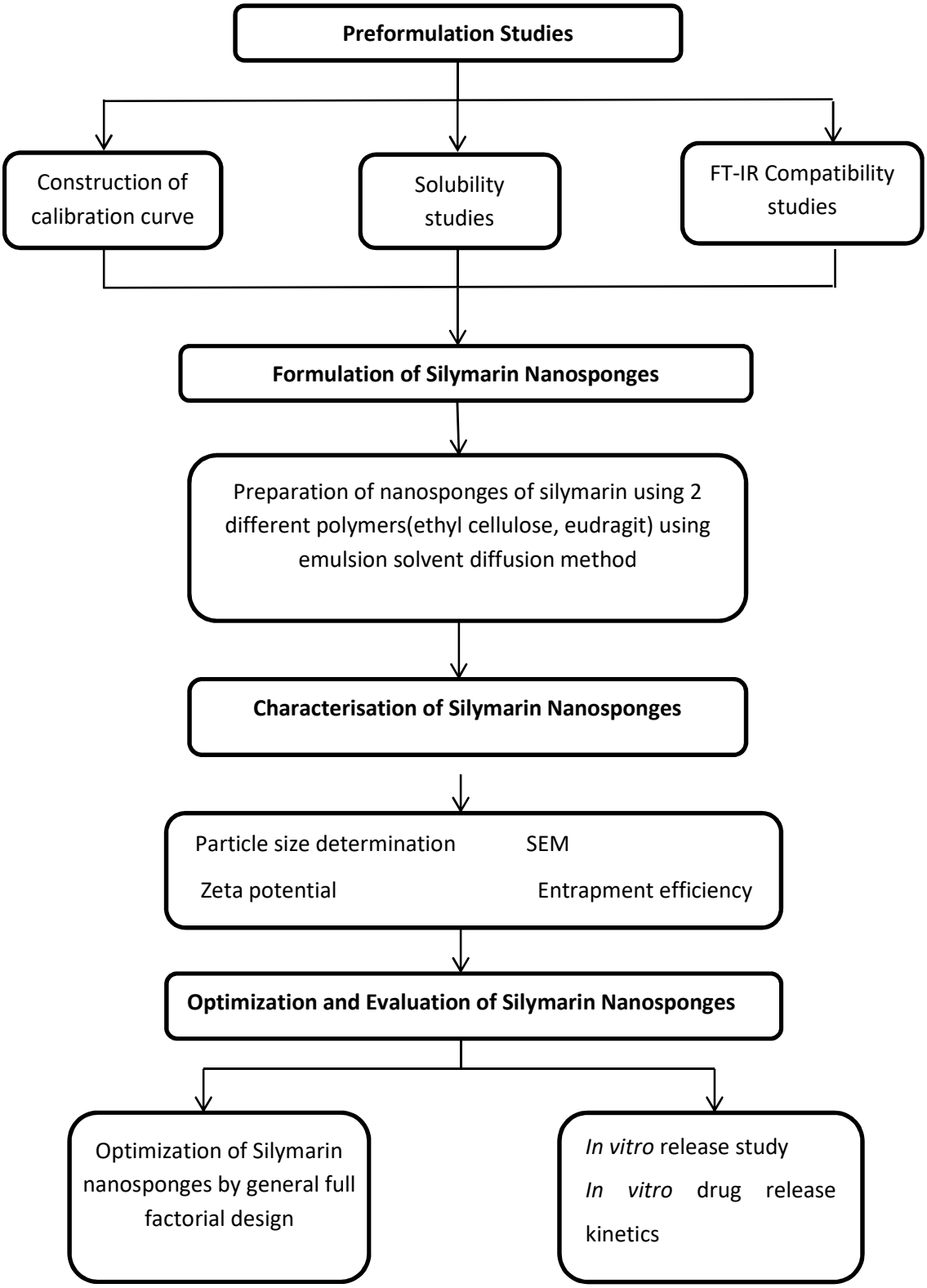
AIM AND OBJECTIVE

- To formulate silymarin nanosponges so as to target cancer cells particularly breast cancer, colorectal cancer using different polymers.
- To increase the bioavailability of silymarin by developing silymarin nanosponges.
- To reduce the dose, dosing frequency and to reduce dose dependent side effects of Silymarin.
- To facilitate drug targeting or selective uptake of drug.
- Deliver the drug at the specified site in a controlled manner without showing any burst effect.

To achieve this objective the following steps were carried out:

- The preformulation studies.
- Formulation of silymarin nanosponges and its optimization by general full factorial design.
- Characterisation of the prepared silymarin nanosponges.
- *In-vitro* evaluation of silymarin nanosponges.

PLAN OF WORK


Preformulation Studies
Construction of calibration curve
Solubility studies
FT-IR Compatibility studies
Formulation of Silymarin Nanosponges
Preparation of nanosponges of silymarin using 2 different polymers(ethyl cellulose, eudragit) using emulsion solvent diffusion method
Characterisation of Silymarin Nanosponges
Particle size determination
SEM
Zeta potential
Entrapment efficiency
Optimization and Evaluation of Silymarin Nanosponges
Optimization of Silymarin nanosponges by general full factorial design
In vitro release study
In vitro drug release kinetics

MATERIALS AND EQUIPMENTS

MATERIALS USED

Sl. No	Materials	Source
1.	Silymarin	Gift sample
2.	Ethyl Cellulose	Himedia, Mumbai
3.	Eudragit	Yarrow Pharma
4.	Polyvinyl Alcohol	Sigma Aldrich
5.	Ethanol	Zhuhai Chemico Industries
6.	Di-Sodium Hydrogen Orthophosphate	SD Fine Chemical Limited
7.	Potassium Dihydrogen Orthophosphate	Qualigens Fine Chemicals, Mumbai

EQUIPMENTS USED

Sl. No	Equipment	Model/ Company
1.	Magnetic Stirrer	REMI-2MLH
2.	Optical Microscope	MOTIC B1 SERIES
3.	UV Spectrophotometer	JASCO V-530
4.	FT-IR Spectrometer	FTIR JASCO -4100
5.	pH Meter	pH TESTER 1,2(EUTECH)
6.	Zeta Sizer	MALVERN
7.	SEM	HITACHI X650, Tokyo, Japan
8.	Dialysis membrane 50mm	Himedia, Mumbai

SOFTWARE USED

Minitab 18 : Minitab INC, USA

DD Solver : Microsoft

DRUG PROFILE

SILYMARIN

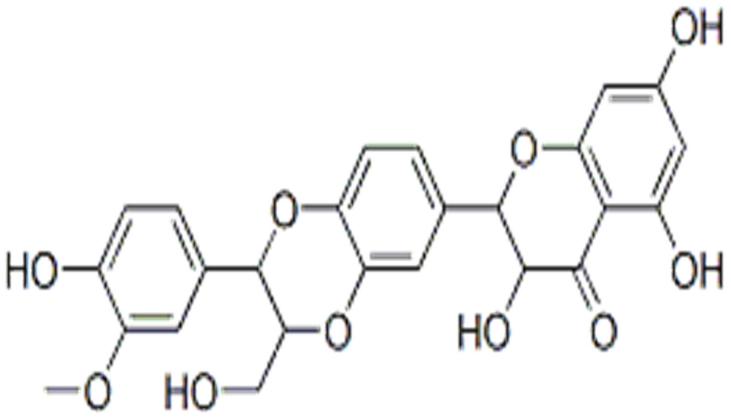
Description:

Silymarin is an active component obtained from the plant *Silybum marianum*, commonly known as milk thistle from the family *Asteraceae*. About 70-80% of the plant consists of silymarin flavinolignan i. e silidianin, silychristin, silybin A and B and iso silybin A and B.

IUPAC name: 2-(2,3-Dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxyl methyl)-1,4-benzodioxin-6-yl)-2,3-dihydro-3,5,7-trihydroxy-4H-1-benzopyean-4-one.

Empirical formula: $C_{25}H_{22}O_{10}$

Chemical structure:



Molecular weight: 482.436

Melting point: 164-174°C

Boiling point: 793°C at 760mm Hg

Density: 1.527g/cm^3

Colour: light yellow powder

Solubility: solubility of silymarin in various solvents is given in Table 2.

Table 2: Solubility of Silymarin in Various Solvents

Transcutol	350.1 mg/ Ml
Ethanol	225.2 mg/ mL
Polysorbate 20	131.3 mg/ mL
Glyceryl monooleate	33.2 mg/ mL
Water	0.04 mg/ mL

Categories:

Hepatoprotective, antioxidative, anti -inflammatory, neuroprotective, neurotropic, antilipid peroxidative, cardioprotective and membrane stabilizing agent.

Mechanism of Action:

- Silymarin due to its property of free radical scavenging can act against lipid per oxidation and also helps to increase cellular content of Glutathione.
- During xenobiotic damage it regulates membrane permeability resulting in better membrane stability.
- Silymarin has asteroid like effect which helps in regulating nuclear expression. The conversation of stellate hepatocytes to myofibroblast which is known factor for cirrhosis is inhibited by silymarin.

Adverse Reactions:

Nausea, diarrhoea, indigestion, flatulence, bloating, fullness or pain, anorexia, occasional laxative effects. It is well-known to cause allergic response in people who are sensitive to *Asteraceae/ Compositae* family.

Dosage and administration:

Oral; hepatic diseases: adult 70-140mg bid/tid

Absorption:

Absorption of silymarin is comparatively low after oral administration i.e about 20-30%. Peak plasma concentrations are achieved within 4-6 hrs.

Metabolism:

In the liver silymarin gets conjugated with sulphates and glucuronic acid and then conjugates passes through plasma and bile.

Elimination:

Silymarin is mainly excreted in bile and to lesser extent in urine. The elimination half -life is silymarin is 1-3 hrs.

Indications:

Silymarin is used to treat hepatitis, as an adjunctive therapy in the treatment of cirrhosis, jaundice and also in alcohol related liver diseases. It has also found to possess anticancer activity.

Contraindications:

Silymarin is contraindicated in cases of hypersensitivity, hepatic encephalopathy, and primary biliary cirrhosis and in cases of hypersensitivity to components of silymarin.

Storage and Handling:

Store in a well closed container in a cool, dry and dark place.

Brand names:

Silymarin marketed product given in Table 3.

Table 3: Marketed Products of Silymarin

Brand names	Manufacturer's
C. Hepasil	*Signova*
C. Levalon	*Micro B&B*
C.Levalon	*Serum Intl.*
C.Prohepforte	*LUPIN*
C.Silimar	*Zydus(G.Rem)*
C.Sison	*Dr. Alson Labs*

EXCIPIENT PROFILE

POLYVINYL ALCOHOL

Synonyms

Airvol, elvanol, gohsenol, PVA, vinyl alcohol polymer

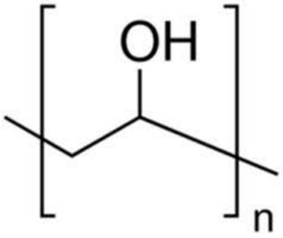
Chemical Name and CAS Registry Number

Ethanol, homopolymer [900-89-5]

Empirical Formula

$(C_2H_4O)_n$

Chemical Structure



Molecular Weight:

20000-200000

Polyvinyl alcohol is a water soluble synthetic polymer represented by formula $(C_2H_4O)_n$. The value of n for commercially available material lies between 500 and 5000, equivalent to a molecular weight range of approximately 20000-200000.

Table 4: Commercially Available Grades of Polyvinyl Alcohol

Grade	Molecular Weight
High viscosity	200000
Medium viscosity	130000
Low viscosity	20000

Functional Category

Coating agent, lubricant, stabilizing agent and viscosity increasing agent.

Application on Pharmaceutical Formulation

Polyvinyl alcohol is used primarily in topical pharmaceutical and ophthalmic formulation. It is used as a stabilizing agent for emulsions (0.25-3.0%w/v). Polyvinyl alcohol is also used as viscosity-increasing agent for viscous formulations such as ophthalmic products. It is used in artificial tears and contact lens solution for lubrication purpose, in sustained-release formulation for oral administration, and in transdermal patches.

Table 5: Uses of Polyvinyl Alcohol

Uses	Concentration
Emulsions	0.5%
Ophthalmic formulations	0.25-3.00%
Topical solutions	2.5%

Description

Polyvinyl alcohol occurs as an odourless, white to cream-colored granular powder.

Typical Properties

Melting point:

- 228ºC for fully hydrolysed grades
- 180ºC-190ºC for partially hydrolysed grades

Refractive Index

n_D^{25}=1.49-1.53

Solubility

PVA is soluble in water and insoluble in organic solvents. Dissolution requites dispersion (wetting) of the solid in water at room temperature followed by heating the mixture to about 90°C for approximately 5 mins. Mixing should be continued while the heated solution is cooled to room temperature.

Specific Gravity

- 1.19-1.31 for solid at 25ºC
- 1.02 for 10% w/v aqueous solution at 25ºC

Viscosity (Dynamic)

Table 6: Viscosity of Commercial Grades of Polyvinyl Alcohol

Grade	Dynamic Viscosity of 4%w/v Aqueous Solution at 200°C (Mpas)
High viscosity	40.0-65.0%
Medium viscosity	21.0-33.0%
Low viscosity	4.0-7.0%

Stability and Storage Conditions

Polyvinyl alcohol is stable when stored in tightly sealed container in a cool, dry place. Aqueous solutions are stable in corrosion resistant sealed containers. Preservatives may be added to the solution if extended storage is required. Polyvinyl alcohol undergoes slow degradation at 100ºC and rapid degradation at 200ºC; it is stable on exposure to light.

Incompatibilities

Polyvinyl alcohol undergoes reactions typical of a compound with secondary hydroxyl groups such as esterification. It decomposed in strong acid and softens or dissolves in weak acids and alkalis. It is incompatible at high concentration at high concentration with inorganic salts, especially sulphates and phosphates, precipitation of polyvinyl 5% w/v can be caused by phosphates, gelling of polyvinyl alcohol solution may occur if borax is present .

Method of Manufacture

Polyvinyl alcohol is produced through the hydrolysis of polyvinyl acetate. The repeating unit of vinyl alcohol is not used as the starting material because it can't be obtained in the quantities and the purity required for polymerization purposes. The hydrolysis proceeds rapidly in methanol, ethanol, or a mixture of alcohol and methyl acetate, using alkalis or mineral acids or catalysis.

Safety

Polyvinyl alcohol is generally considered a non-toxic material. It is non-irritant to the skin and eyes at concentration up to 10% concentration upto 7% are used in cosmetics.

ETHYL CELLULOSE

Nonproprietary names

BP : Ethylcellulose

PhEur : Ethylcellulosum

USPNF : Ethylcellulose

Synonyms

Aquacoat; E462; Ethocel; Surlease

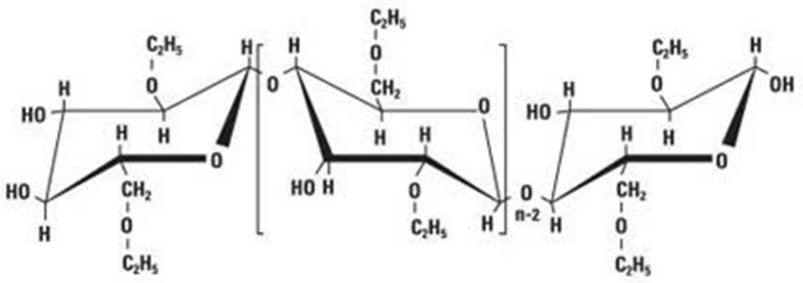
Chemical names and CAS registry number

Cellulose ethyl ether [9004-57-3]

Empirical formula

Ethyl cellulose is an ethyl ether of cellulose, a long chain polymer consisting of anhyroglucose units joined by acetate linkage. Each anhydroglucose units has three replacable hydroxyl groups which are substituted to the extent of 2.25-2.60 ethoxul groups(OC_2H_5) per unit, equivalent to an ethoxyl content of 44-51%

Structural formula



Functional category

Coating agent; tablet binder; viscosity increasing agent

Applications in pharmaceutical formulation or technology

Ethyl cellulose is widely used in oral and topical pharmaceutical formulations. The main use of ethyl cellulose in oral formulations is as a hydrophobic coating agent for tablet and granules.

Ethyl cellulose coatings are used to modify the release of a drug, to mask an unpleasant taste, or to improve the stability of the formulations. Higher viscosity ethyl cellulose grades tend to produce stronger, tougher films. Ethyl cellulose is

also widely used in drug microencapsulation, high viscosity grades usually being used.

Description

Ethyl cellulose is a tasteless, free flowing, white or light tan coloured powder.

Typical properties

Density:

$0.4 g/cm^3$

Glass transition temperature:

$130\text{-}133^0C$

Melting point:

$165\text{-}173^0C$

Hygroscopicity:

Ethyl cellulose absorbs very little water at high relative humidity or during immersion.

Solubility:

Practically insoluble in glycerine, propylene glycol and water. Ethyl cellulose that contain not less than 46.5% of ethoxyl group is freely soluble in chloroform, ethanol, ethyl acetate, methanol and toluene.

Specific gravity: 1.12-1.15

Viscosity:

Various grades of ethyl cellulose are commercially available having viscosities ranging from 3-385MPas.

Stability and storage conditions

Ethyl cellulose is a stable, slightly hygroscopic material. It is chemically resistant to alkalis, both dilute and concentrated, and to salt solutions, though it is more sensitive to acidic materials than cellulose esters. Ethyl cellulose is subject to oxidative degradation in the presence of sunlight or UV light at elevated temperatures.

The bulk material must be stored in a dry place, in a well closed container at a temperature between $7\text{-}32^0C$.

Incompatibilities

Incompatible with paraffin wax and microcrystalline wax.

Method of manufacture

Ethyl cellulose is prepared from wood pulp by treatment with alkali followed by ethylation of alkali cellulose with chloroethane.

Safety

Ethyl cellulose is widely used in oral and topical pharmaceutical formulations. It is also used in food products. It is generally regarded as a nontoxic, non-allergenic and non-irritant material.

Handling precautions

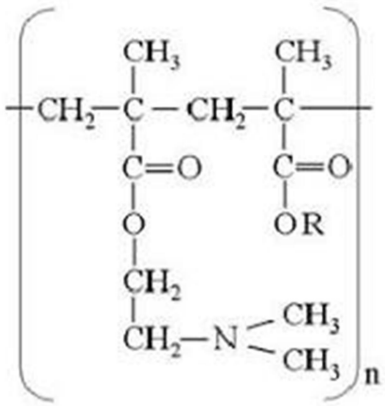
Observe normal precautions appropriate to the circumstances and quantity of materials handled. Dust may be irritant to the eyes and eye protectant should therefore be worn. Ethyl cellulose is combustible.

EUDRAGIT

Chemical name: methacrylic acid

Chemical formula: $C_4H_6O_2$

Chemical Structure:



R = CH_3, C_4H_9

Synonyms:

- 2-(methoxycarbonyl)-1-propene
- 2-methyl-2-propenoic acid methyl ester

Appearance:

Colourless

Odour:

Unpleasant, acrid and repulsive.

Solubility:

Soluble in warm water

Miscible with most organic solvents

Density:

1.02g/cm^3

Molar mass:

86.06g/mol

Boiling point:

161^0C

Melting point:

15^0C

Applications

Ophthalmic drug delivery

Buccal and sublingual drug delivery

Gastrointestinal drug delivery

Intestinal drug delivery

Colon drug delivery

Transdermal drug delivery

Vaginal drug delivery

Gene drug delivery

Vaccine drug delivery

Eudragit polymer were selected for this studies because they dissolved at pH > 5.5 and are not soluble in acidic gastric fluid and which can prevent the premature release of the incorporated drug molecule during the preparation before dosing.

Eudragit are sufficiently being used in pre-clinical models to enable *in vivo* safety pharmacology studies where small changes in organ function and physiology are assessed during lead optimization.

Method of Manufacture

In one route, acetone cyanohydrin is converted to methacrylamide sulphate using sulphuric acid which is then hydrolysed to methacrylic acid. In the second route, isobutylene or tert-butanol are oxidised to methacrolein, then methacrylic acid.

EXPERIMENTAL METHODS

I. PREFORMULATION STUDIES:

Physical characteristics:

By visual examination the drug was tested for its physical characters like colour, odour and texture.

Solubility test:

Silymarin powder (about 1mg) was taken in a test tube and solubility in ethanol, water, dichloromethane and chloroform was tested.

Preparation of stock solution

The standard stock solution of silymarin was prepared by transferring accurately weighed quantity (10 mg) of silymarin raw material in 100 ml of volumetric flask. The drug was dissolved in few ml of ethanol and the volume was made up to 100 ml with ethanol to get a stock solution of 100 µg/mL.

Selection of Wavelength

The standard stock solution was scanned in the range of 200 to 400 nm in UV spectrophotometer using phosphate buffer pH 6.8 as blank. The absorption maximum was found at 288 nm.

Construction of calibration curve of silymarin:

From the standard stock solution of silymarin 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 ml were withdrawn to 10 ml volumetric flask and then made up volume with phosphate buffer pH 6.8 to get a concentration range of 5-40 µg/mL. The absorbance of these solutions was measured at 288nm using JASCO V-530 UV 1600 UV- visible spectrophotometer. Phosphate buffer pH 6.8 was used as blank. The calibration curve was plotted between concentration and absorbance [63].

Preparation of Buffer Solutions

Phosphate buffer pH 6.8: An accurately weighed quantity of 28.80gm of disodium hydrogen phosphate and 11.45gm of potassium dihydrogen phosphate was dissolved in sufficient water to produce 1000ml.

Drug Excipient Compatibility Studies

FT-IR spectrum of drug was recorded using FT-IR Spectro photometer (Shimadzu JASCO 4100). The diffuse reflectance technique was utilised in the mid IR 4000-400 cm^{-1} spectral region. The procedure consist of dispersing the sample in KBr(100mg) using a mortar, triturating the materials into a fine powder bed into the holder using compression gauge. The pressure was around 5 tons for 5 minutes. The pellet was placed in the light path and the spectrum was recorded. The characteristic peaks of the functional groups were interpreted.

The FTIR spectrum of silymarin, polymers ethyl cellulose and eudragit were recorded. The spectrum of physical mixture of silymarin, polymer and co-polymer were also documented to check for their compatibility.

II. FORMULATION OF SILYMARIN NANOSPONGES BY EMULSION SOLVENT DIFFUION METHOD:

Emulsion solvent diffusion method was used to formulate silymarin loaded nanosponges by using a suitable polymer. Dispersed phase consists of specified amount of drug and polymer which was dissolved in 20 ml of an organic solvent, dichloromethane. Aqueous phase consists of specified amount of poly vinyl alcohol dissolved in 100 ml distilled water. Disperse phase was added drop by drop into aqueous phase by stirring on magnetic stirrer at 1000 rpm for about 2 hours. The nanosponges formed were collected by filtration and dried in oven at 40°C for about 24 hours. They were then kept in the vacuum desiccators to remove the residual solvent. The silymarin nanosponges were formulated using polymers ethyl cellulose and eudragit [30].

III. CHARACTERIZATION OF NANOSPONGES

FTIR Spectroscopy of Nanosponges

Before formulating a drug substance into dosage form, it is essential that it should be chemically and physically compatible. Compatibility studies give information needed to define the nature of the drug substance and provide a frame work for the drug combination with pharmaceutical excipients in the fabrication of a dosage form. This study was carried out by using infrared spectrophotometer to find if there is any possible chemical interaction between the silymarin and polymers.

A few mg of sample (silymarin nanosponges) was weighed and mixed with 100 mg of potassium bromide (dried at 40-50°C). The mixture was taken and compressed under 10- ton pressure in hydraulic press to form a pellet. The pellet was scanned from 4000-400 cm^{-1} in IR spectrophotometer.

Determination of Percentage Yield

Silymarin loaded nanosponges were weighed after drying. Percentage yield was calculated by

$$\%\ yield = \frac{Practical\ weight\ of\ nanosponges\ obtained}{Theoretical\ weight(\ drug + polymers)} \times 100$$

Scanning Electron Microscopy (SEM)

SEM analysis was performed to determine their microscopic characters (shape & morphology) of prepared silymarin nanosponges. Nanosponges were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy (Hitachi X650, Tokyo, Japan) in different magnifications .Samples were placed on glass slide kept under vacuum and then by using sputter coater unit, samples were coated with a thin gold layer, operated at 15kv acceleration voltage [30].

Particle Size Determination

The average mean diameter and size distribution of loaded nanosponges is found by Dynamic Light Scattering method using Malvern zeta sizer at 25°C.The

dried nanosponges were dispersed in water to obtain proper light scattering intensity for silymarin nanosponges [43].

Determination of Zeta Potential

Zeta potential is a measure of surface charge. The surface charge (electrophoretic mobility) of nanosponge can be determined by using Zeta sizer (Malvern Instrument) having zeta cells, polycarbonate cell with gold plated electrodes and using water as medium for sample preparation. It is essential for the characterisation of stability of the nanosponges [45].

Determination of Entrapment Efficiency

The entrapment efficiency of nanosponges were determined by adding 10 ml of phosphate buffer of pH 6.8 and sonicated in a bath sonicator and filtered. 1 ml of filtrate is made up to 10 ml with phosphate buffer and was assayed spectrophotometrically at 288 nm (UV visible spectrophotometer, model UV-1601 PC, Shimadzu). The amount of entrapped drug was calculated from the equation [43].

$$Entrapment\ Efficiency\ = \frac{Practical\ drug\ content}{Theoretical\ drug\ content}\ \times 100$$

***In Vitro* Release Studies**

Drug release was determined by dialysis method; two ml of each formulation (test and control) were poured into dialysis bags and put into 25 ml phosphate buffer (pH 6.8) and stirred (100 rpm, room temperature). At predetermined time intervals, 2 ml of phosphate buffer was taken and then substituted by fresh phosphate buffer. Finally, the amounts of released silibinin in phosphate buffer were measured by spectrophotometer at 288 nm [40]. Aliquots withdrawn were assayed at each time interval for the drug released at λ_{max} of 288 nm using UV-Visible spectrophotometer by keeping phosphate buffer pH 6.8 as blank and the amount of released drug was estimated by the standard curve[30].

Optimization

The formulation of silymarin nanosponges is optimized by general full factorial design. The type of polymer and concentration of polymer are considered as independent variables and the percentage drug released is considered as

dependent variable. Minitab 18 Software (Minitab Inc, USA) is used for this purpose.

***In Vitro D*rug Release Kinetics**

The drug release kinetics of Silymarin nanosponges was determined by plotting the following kinetic models, using the data collected from *in vitro* release studies (zero order, first order and Higuchi equations). The mechanism of drug release was determined by using Korsmeyer-Peppas equations [59].

Zero-Order Kinetics:

Cumulative amount of drug released was plotted against time.

$$C = K_0 t$$

Where K_0 is the zero-order rate constant expressed in units of concentration/time and *t* is the time in hours. A graph of concentration vs. time would yield a straight line with a slope equal to K_0 and intercept the origin of the axis. This kinetics describes concentration independent drug release from the formulations.

First order kinetics:

First order graph is plotted by log cumulative percentage of drug remaining vs. time. This kinetics describes concentration dependent drug release from the formulations.

$$\text{Log } C = \text{Log } Co + K_t / 2.303$$

Where C_0 is the initial concentration of drug, *k* is the first order constant, and *t* is the time.

Higuchi's Model:

Higuchi's model as cumulative percentage of drug released vs. square root of time.

$$Q = Kt_{1/2}$$

Where *K* is the constant reflecting the design variables of the system and *t* is the time in hours. This model describes the release of drug on the basis of Fickian diffusion as a square root of time dependent process from swellable matrix.

Korsmeyer-Peppas Equations:

The mechanism of drug release, the first 60% of drug release were plotted in Korsmeyer et al's equation log cumulative percentage of drug released vs. log time, and the exponent *n* was calculated through the slope of the straight line,

$Mt / M\infty = Kt^n$

Where $Mt/M\infty$ is the fractional solute release, t is the release time, K is a kinetic constant characteristic of the drug/polymer system, and n is an exponent that characterizes the mechanism of release of tracers. This type of drug release is controlled by combination of polymer swelling, erosion and diffusion through hydrated matrix. The mechanism of diffusion is identified from the values of 'n'.

- ✓ The value of $n \leq 0.45$ indicating fickian diffusion(Case I)
- ✓ The value of n between 0.45 to 0.89($0.45<n<0.89$) indicating non fickian (anomalous) diffusion. Here release is controlled by combination of diffusion and polymer relaxation.
- ✓ The value of n =0.89, indicating the zero order release or case 2 transport. Here the drug release rate is independent of time and involves polymer relaxation.
- ✓ The value of $n > 0.89$, indicating the super case 2 transport.

Kinetic study was performed using DD solver software.

RESULTS AND DISCUSSION

I. PREFORMULATION STUDIES

Physical Characteristics

Silymarin was checked for its colour, odour and texture. Silymarin is light yellow coloured powder in appearance, odourless and amorphous in nature.

Solubility

Solubility test for Silymarin was carried out in different solvents such as ethanol, water, dichloromethane and chloroform and results are given in Table 7.

Table 7: Solubility test for Silymarin in different solvents

Sl. No	Solvent	Soluble	Sparing Soluble	Insoluble
1.	Ethanol	✓	-	-
2.	Dichloromethane	✓	-	-
3.	Chloroform	-	✓	-
4.	Water	-	-	✓

Selection of Wavelength

The Silymarin stock solution of concentration 100µg/mL was scanned in the range of 200-400nm for $\lambda_{max.}$ using double beam UV Spectrophotometer. The absorption peak obtained is shown in Figure 4.

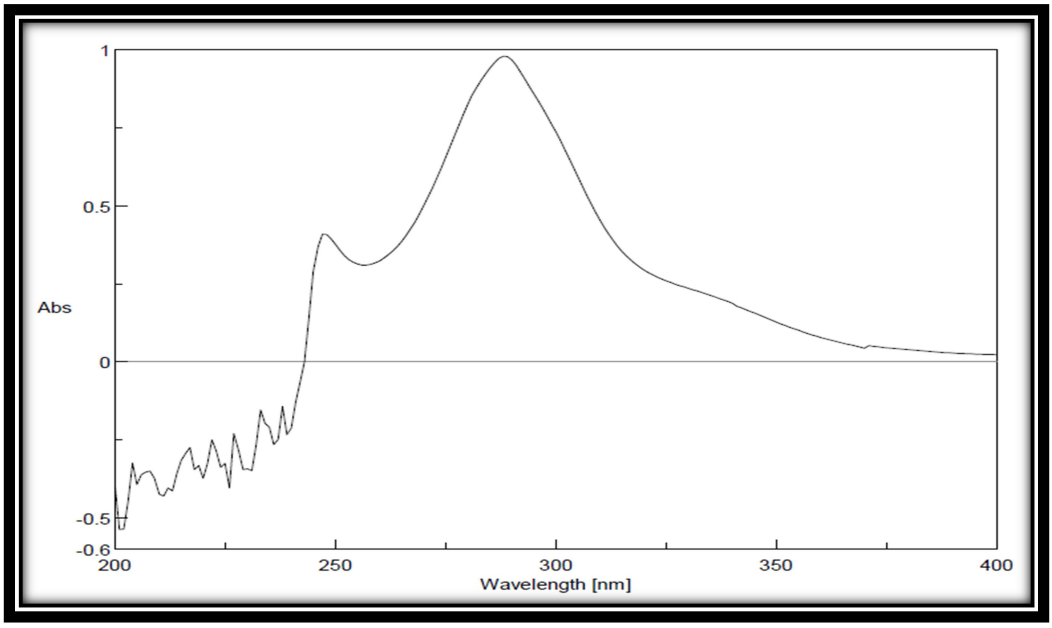


Figure 4: UV spectra of Silymarin

The maximum absorption of Silymarin was found to be at 288nm and hence it is selected as the wavelength for further studies.

Construction of calibration curve of Silymarin

In the calibration curve, linearity was obtained between 5-40 µg/ml concentration of Silymarin and the regression value was found to be r^2 = 0.9996. Hence we can conclude that Silymarin obeys Beer Lambert's Law at the concentration between 5-40 µg/ml. The results are shown in Table 8 and Figure 5.

Table 8: Concentration and absorbance values for estimation of Silymarin

Sl. No	Concentration (µg/ml)	Absorbance(AU) at 288nm
1.	5	0.1686
2.	10	0.3624
3.	15	0.5357
4.	20	0.6963
5.	25	0.8770
6.	30	1.0693
7.	35	1.2700
8.	40	1.4516

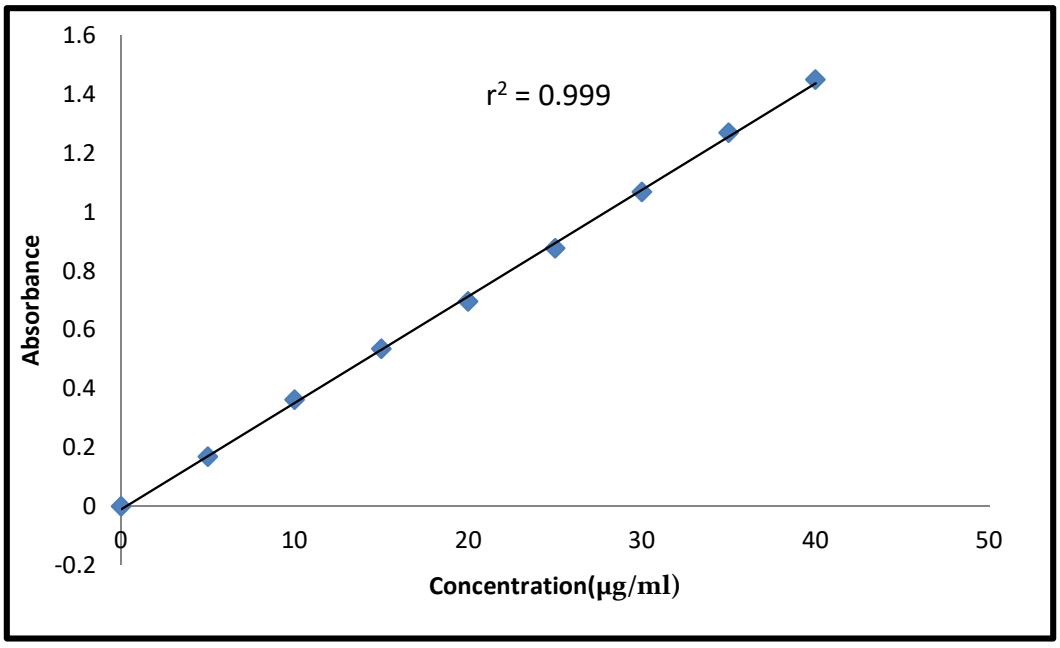


Figure 5: Calibration graph of Silymarin

Excipient Compatibility Studies

Fourier Transform Infrared (FT-IR) spectra of the samples were obtained using a FTIR Jasco 4100 Spectrometer by KBr disc method. The spectrums were recorded for the pure drug and physical mixture of drug and polymer and are shown in Figures 6,7,8,9,10,11and 12.

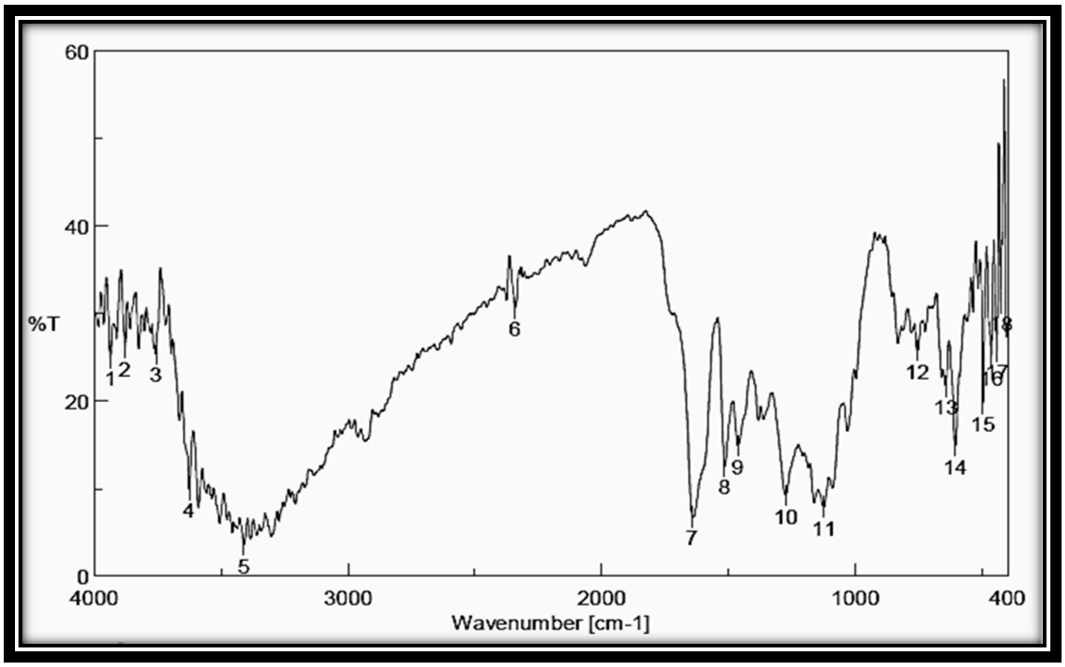


Figure 6: FTIR spectrum of Silymarin

Table 9: FTIR interpretation of Silymarin

Materials	Standard wave number (cm^{-1})	Test wave number (cm^{-1})	Functional group assignment
SILYMARIN	3650-3200	3410.49 3625.52	OH stretching
	1820-1665	1643.05	C=O stretching
	1320-1210	1273.75	C-O-C stretching
	1161-1029	1121.4	In plane =C-H bending

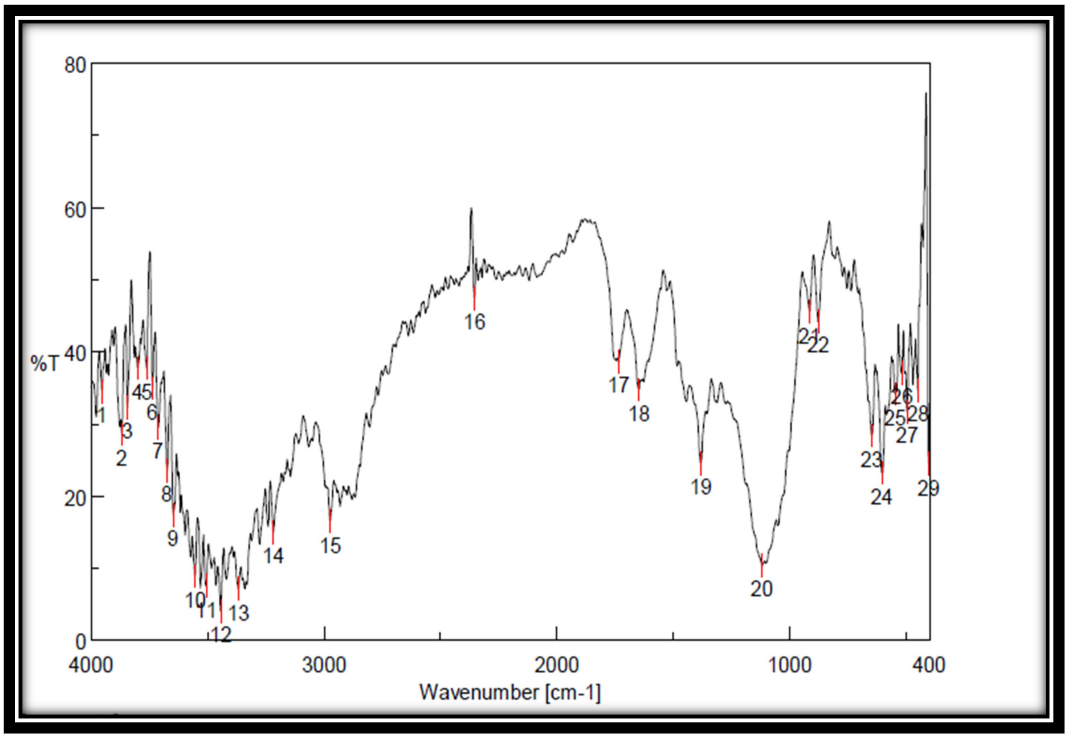


Figure 7: FTIR spectrum of Ethyl Cellulose

Table 10: FTIR interpretation of Ethyl Cellulose

Materials	Standard wave number (cm^{-1})	Test wave number (cm^{-1})	Functional group assignment
ETHYL CELLULOSE	3650-3200	3647.7	O-H stretching
	2960-2850	2974.66	C-H stretching
	1820-1665	1734.66	C=O stretching
	1680-1620	1647.88	C=C stretching
	1300-1000	1118.51	C-O stretching

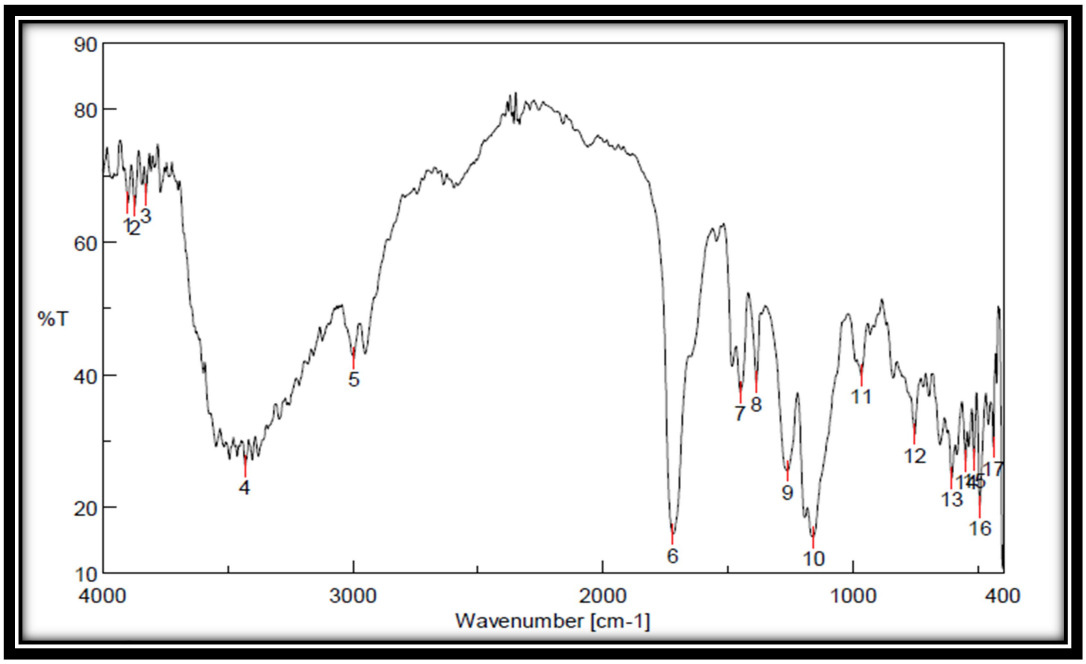


Figure 8: FTIR spectrum of Eudragit

Table 11: FTIR interpretation of Eudragit

Materials	Standard wave number (cm^{-1})	Test wave number (cm^{-1})	Functional group assignment
EUDRAGIT	3000-3700	3430.74	O-H stretching
	1500-1800	1720.19	N-H bending
	2700-3300	2995.87	C-H stretching
	1300-1500	1451.17 1386.57	C-H bending
	1000-1300	1262.18 1159.01	C-O stretching

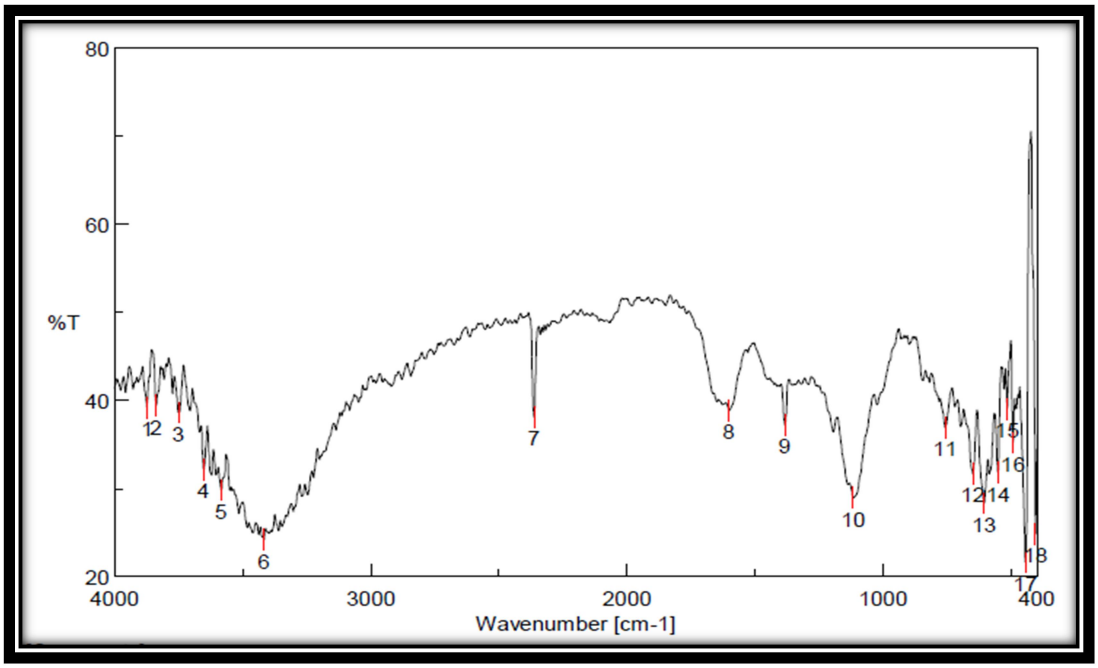


Figure 9: FTIR spectrum of Poly Vinyl Alcohol (PVA)

Table 12: FTIR interpretation of Poly Vinyl Alcohol

Materials	Standard wave number (cm^{-1})	Test wave number (cm^{-1})	Functional group assignment
POLYVINYL ALCOHOL	3300-3600	3584.06	OH stretching
	2850-2970	2862.37	CH_3 stretching
	1500-1760	1600.63	COOH
	1340-1470	1383.68	Alkanes bending
	1000-1300	1116.58	C-O stretching
	600-800	757.888 648.929	C-H rocking

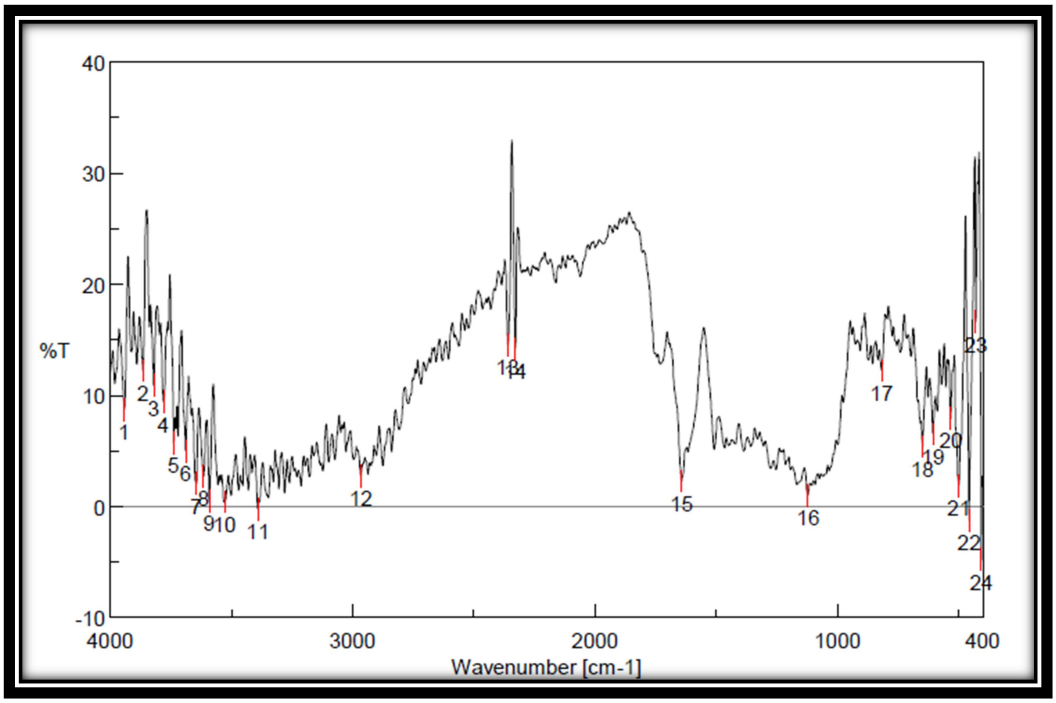


Figure 10: FTIR spectrum of physical mixture containing Silymarin, EC and PVA

Table 13: FTIR interpretation of physical mixture containing Silymarin, EC and PVA

Materials	Standard wave number (cm^{-1})	Test wave number (cm^{-1})	Functional group assignment
MIXTURE CONTAINING SILYMARIN, PVA and EC	3650-3200	3644.88 3613.95	OH stretching
	2970-2850	2965.98	C-H stretching
	1725-1665	1643.05	C=O stretching
	1320-1210	1273.75	C-O-C stretching
	1161-1029	1124.3	In plane =C-H bending
	800-600	816.706 648.929	C-H rocking

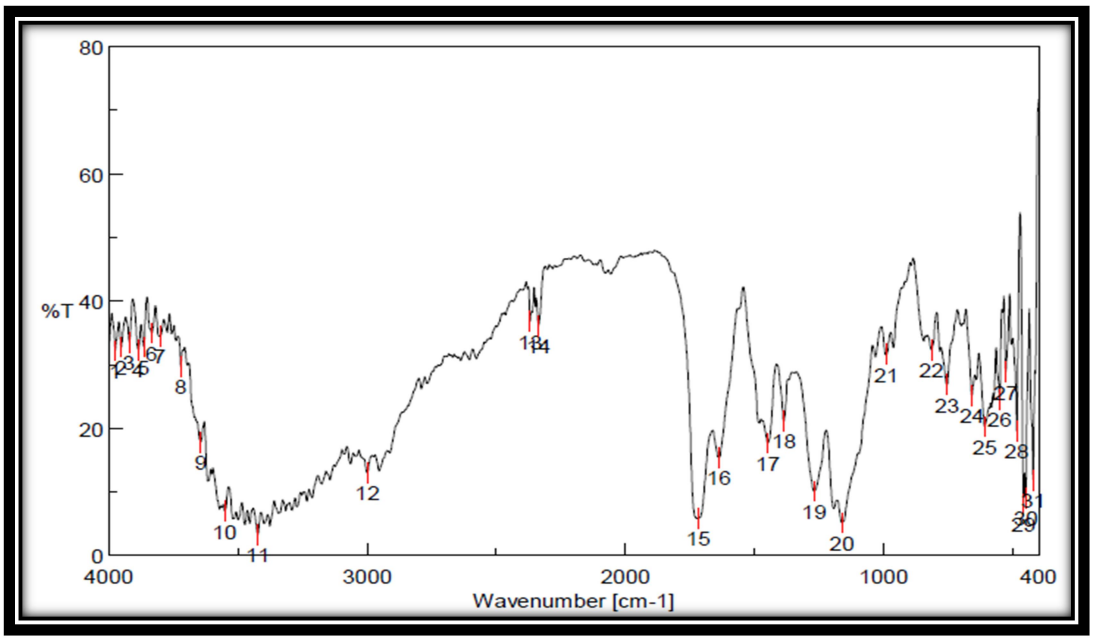


Figure 11: FTIR spectrum of physical mixture containing Silymarin, Eudragit and PVA

Table 14: FTIR interpretation of mixture containing Silymarin, Eudragit and PVA

Materials	Standard wave number (cm^{-1})	Test wave number (cm^{-1})	Functional group assignment
MIXTURE CONTAINING SILYMARIN, EUDRAGIT and PVA	3650-3200	3642.87 3423.033	OH stretching
	3300-2700	2999.73	C-H stretching
	1820-1665	1718.26	C=O stretching
	1800-1500	1639.2	N-H bending
	1500-1300	1386.57	C-H bending
	1320-1210	1268.93	C-O-C stretching
	1161-1029	1161.9	In plane =C-H bending
	800-600	814.777 658.571	C-H rocking

The peaks present in the FTIR spectra of pure Silymarin are present in the FTIR spectra of physical mixture containing Silymarin with ethyl cellulose and Silymarin with eudragit. It is therefore evident that the Silymarin is compatible with the excipients ethyl cellulose, eudragit and poly vinyl alcohol and can be chosen for the formulation of Silymarin nanosponges.

II. FORMULATION OF NANOSPONGES

Selection of polymers for the formulation of Silymarin nanosponges by emulsion solvent diffusion method was based on the trial batches carried out by using different polymers such as ethyl cellulose, eudragit, sodium alginate, HPMC, carbopol, hydroxyl ethyl cellulose, chitosan and pectin and the details are depicted in Table 15. Drug: polymer ratio was selected based on the literature. The results indicated that ethyl cellulose and eudragit was found to be suitable for the formulation of Silymarin nanosponges.

Table 15: Trial batches for formulation of Silymarin nanosponges

Drug	Polymer	Ratio	Result Observed
SILYMARIN	Ethyl cellulose	1:2	Product obtained
	Eudragit	1:2	Product obtained
	Hydroxy propyl methyl cellulose	1:2	Less yield
	Hydroxyl ethyl cellulose	1:2	Less yield
	Carbopol	1:2	Gel like product
	Sodium alginate	1:2	Gel like product
	Chitosan	1:2	No product
	Cyclodextrin	1:2	No yield
	Pectin	1:2	No product

Total ten formulations (F1-F5 and F6-F10) of Silymarin nanosponges with two different polymers ethyl cellulose and eudragit in different ratios were formulated by emulsion solvent diffusion method as given in Table 16 and Table 17.

Table 16: Formulation of Silymarin nanosponges

S. No	Formulation code	Drug	Polymer	Drug : polymer ratio
1	F1	SILYMARIN	Ethyl cellulose	1:0.5
2	F2		Ethyl cellulose	1:1
3	F3		Ethyl cellulose	1:1.5
4	F4		Ethyl cellulose	1:2
5	F5		Ethyl cellulose	1:3
6	F6		Eudragit	1:0.5
7	F7		Eudragit	1:1
8	F8		Eudragit	1:1.5
9	F9		Eudragit	1:2
10	F10		Eudragit	1:2.5

Table 17: Formulation of Silymarin nanosponges by emulsion solvent diffusion technique

S. No	Formulation code	Weight of drug (mg)	Weight of polymer (mg)	Weight of poly vinyl alcohol (mg)
1	F1	100	50	200
2	F2	100	100	200
3	F3	100	150	200
4	F4	100	200	200
5	F5	100	300	200
6	F6	100	50	200
7	F7	100	100	200
8	F8	100	150	200
9	F9	100	200	200
10	F10	100	250	200

III. CHARACTERISATION OF SILYMARIN NANOSPONGES

FTIR Spectroscopy of Silymarin Nanosponges

FTIR spectrum of Silymarin nanosponges using ethyl cellulose is given in Figure12.

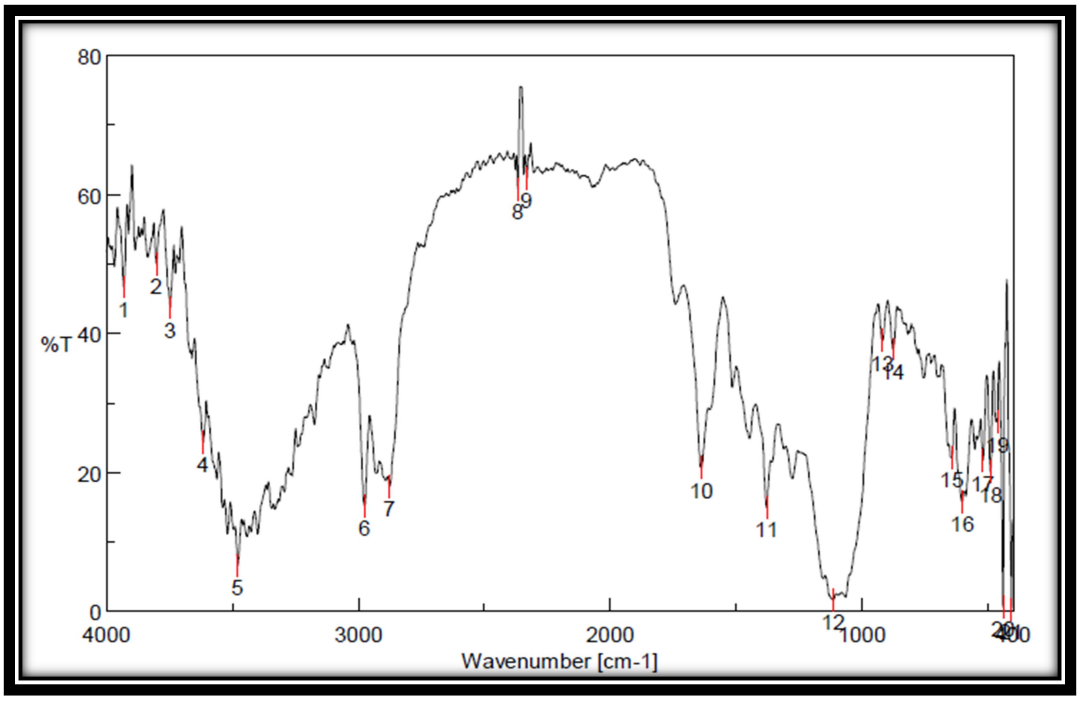


Figure 12: FTIR spectrum of Silymarin nanosponges using EC

Table 18: FTIR interpretation of Silymarin nanosponges using EC

Materials	Standard wave number (cm^{-1})	Test wave number (cm^{-1})	Functional group assignment
FORMULATION F4	3650-3200	3615.88 3478.95	OH stretching
	2970-2850	2876.31	C-H stretching
	1725-1665	1668.23	C=O stretching
	1161-1029	1114.65	In plane =C-H bending
	800-600	876.488 643.144	C-H rocking

FTIR spectrum of Silymarin nanosponge using eudragit is given in Figure 13.

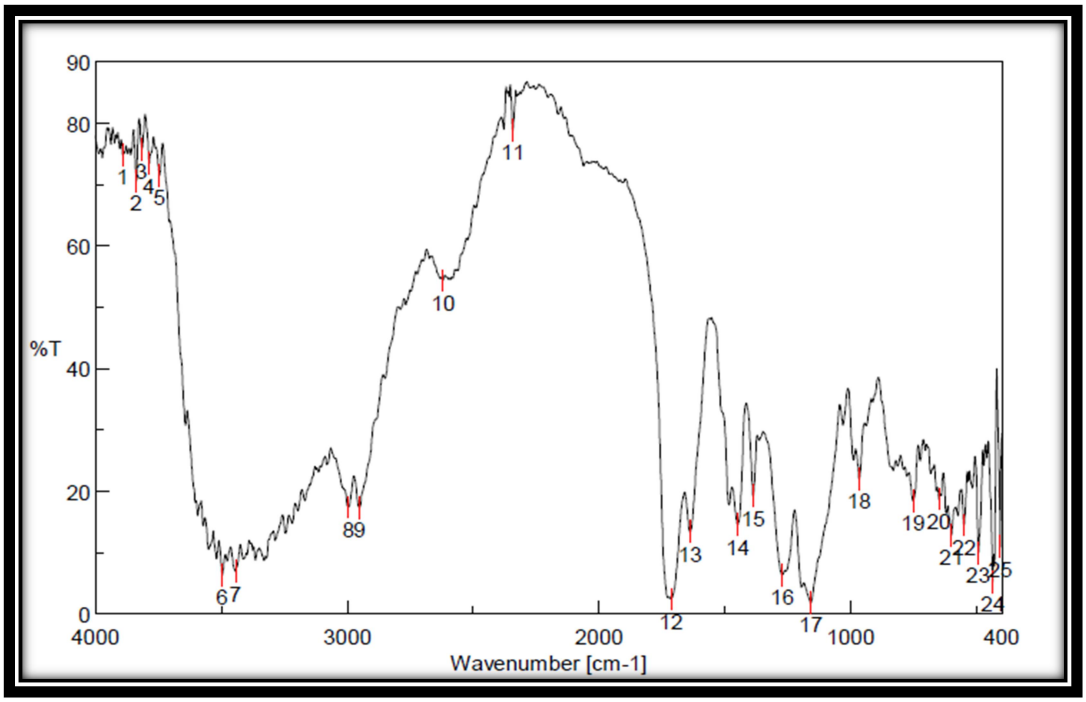


Figure 13: FTIR spectrum of Silymarin nanosponges using Eudragit

Table 19: FTIR interpretation of Silymarin nanosponges using Eudragit

Materials	Standard wave number (cm^{-1})	Test wave number (cm^{-1})	Functional group assignment
FORMULATION F9	3650-3200	3497.27 3444.24	OH stretching
	3300-2700	2993.94 2951.52	C-H stretching
	1820-1665	1714.41	C=O stretching
	1800-1500	1638.23	N-H bending
	1500-1300	1449.24	C-H bending
	1320-1210	1271.82	C-O-C stretching
	1161-1029	1159.01	In plane =C-H bending
	800-600	753.066 648.929	C-H rocking

The peaks present in the FTIR spectra of pure Silymarin are present in the FTIR spectra of formulations F3 and F9. The FTIR interpretations indicated that the Silymarin is compatible with the excipients ethyl cellulose, eudragit and poly vinyl alcohol and no interactions observed in all formulations of nanosponges.

Percentage yield analysis

Percentage yield of the formulated Silymarin nanosponges were calculated using the formula:

$$Percentage\ Yeild = \frac{Practical\ yield}{Theoritical\ yield} \times 100$$

Table 20: Percentage yield of Silymarin nanosponges

S. No	Formulation code	Percentage yield (%)
1.	F1	65.76
2.	F2	71.25
3.	F3	73.28
4.	F4	78.16
5.	F5	80.34
6.	F6	32.08
7.	F7	34.14
8.	F8	48.57
9.	F9	49.75
10.	F10	57.22

The percentage yield was minimum for formulation F6 (32%) and maximum for formulation F5 (80.34%). From the results we can conclude that as the concentration of polymer increases the percentage yield also increases. It can also be noted that the yield obtained while using ethyl cellulose as polymer is much higher when compared with eudragit. The percentage yield of all formulations is depicted in Figure 14.

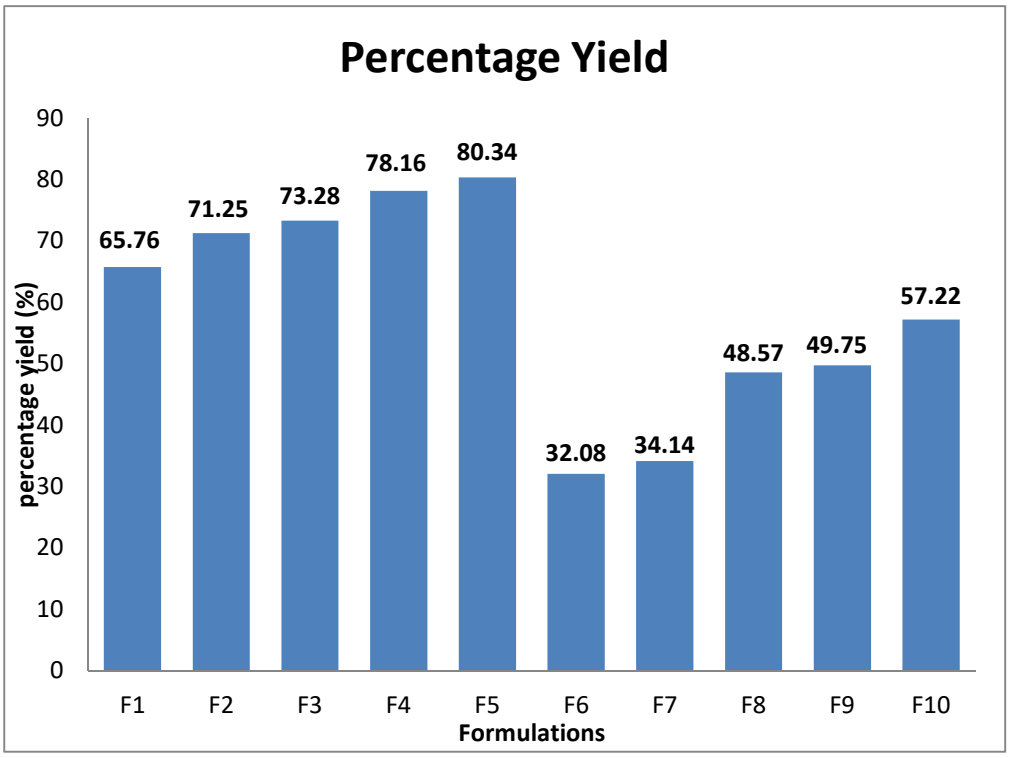


Figure 14: Percentage yield analysis of Silymarin nanosponges

Scanning Electron Microscopy

SEM analyses of the formulated Silymarin nanosponges were performed to evaluate the surface morphology of nanosponges. The SEM images of formulations F4 and F9 are shown in Figure 15 and Figure 16 respectively.

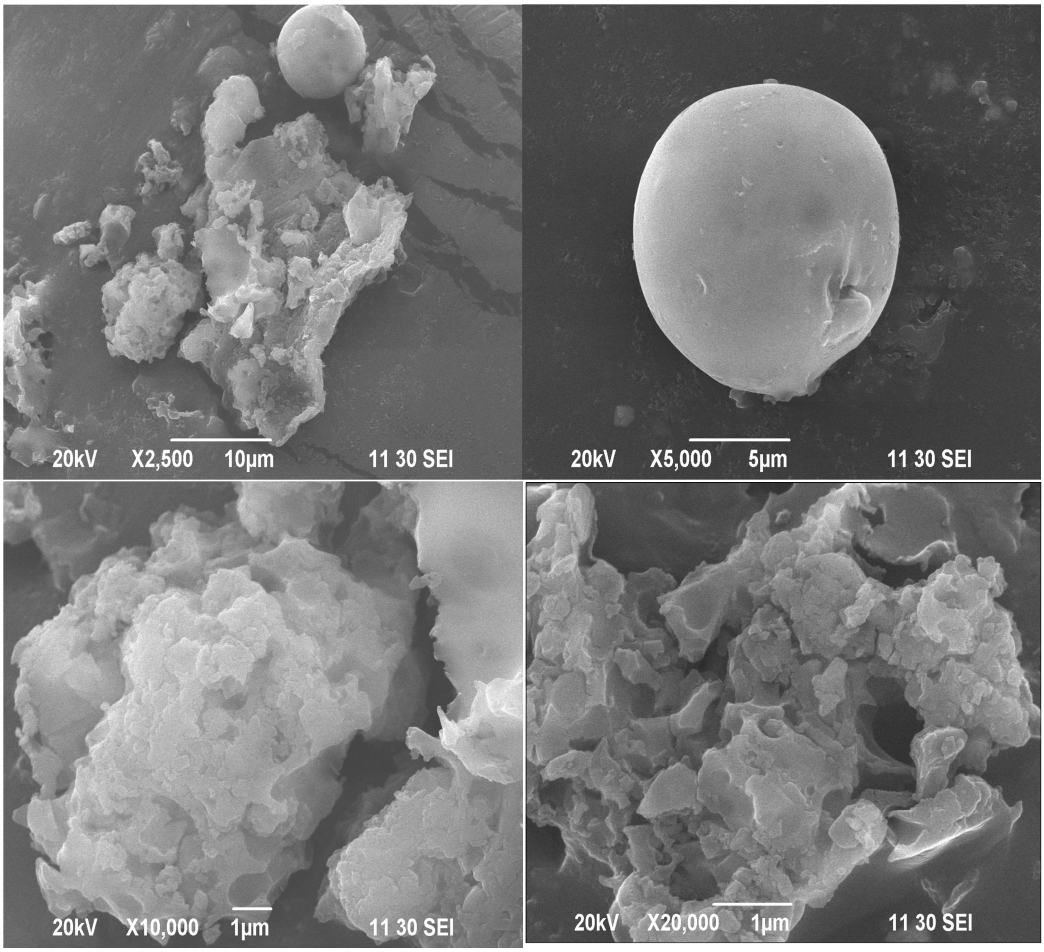


Figure 15: SEM images of Silymarin nanosponges using Ethyl Cellulose

SEM images showed the nanosponge was porous with a smooth surface morphology and spherical in shape. Due to evaporation of solvent, the nanosponge shell found to be smooth porous where outer surface was shiny smooth and inner surface was porous. The spongy and porous nature of the nanosponges can be seen in the above figures. The presence of pores was due to the impression of diffusion of the solvent dichloromethane. These results are in agreement with results obtained by Priyanka *et al.* (2018) [30].

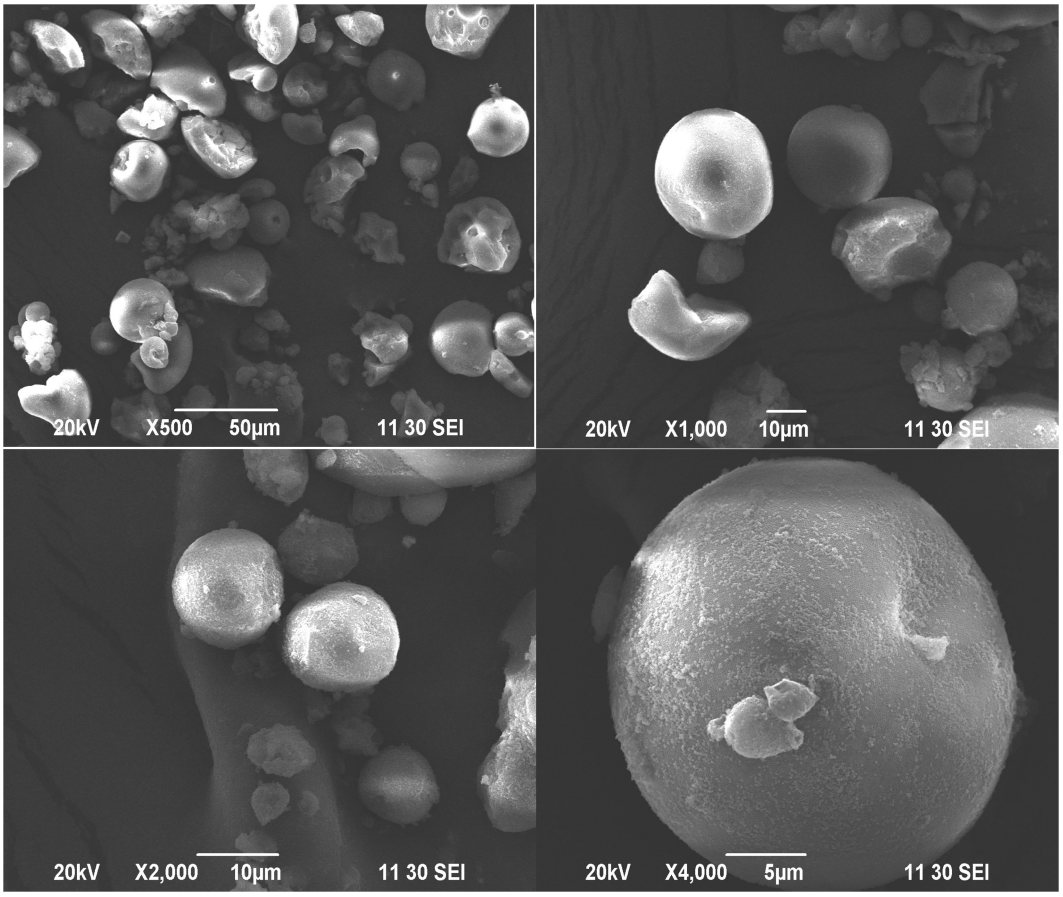


Figure 16: SEM images of Silymarin nanosponges using eudragit

Particle Size Measurement

The particle size is one of the most important parameter for the characterisation of nanosponges. The average particle sizes of the prepared Silymarin nanosponges were measured using Malvern zeta sizer.

Particle size analysis showed that the average particle size of Silymarin nanosponges formulated using ethyl cellulose (F4) was found to be 4097 nm with polydispersity index (PDI) value 1.000 and with intercept 1.41. The zeta size distribution of ethyl cellulose - silymarin nanosponges is depicted in Figure 17.

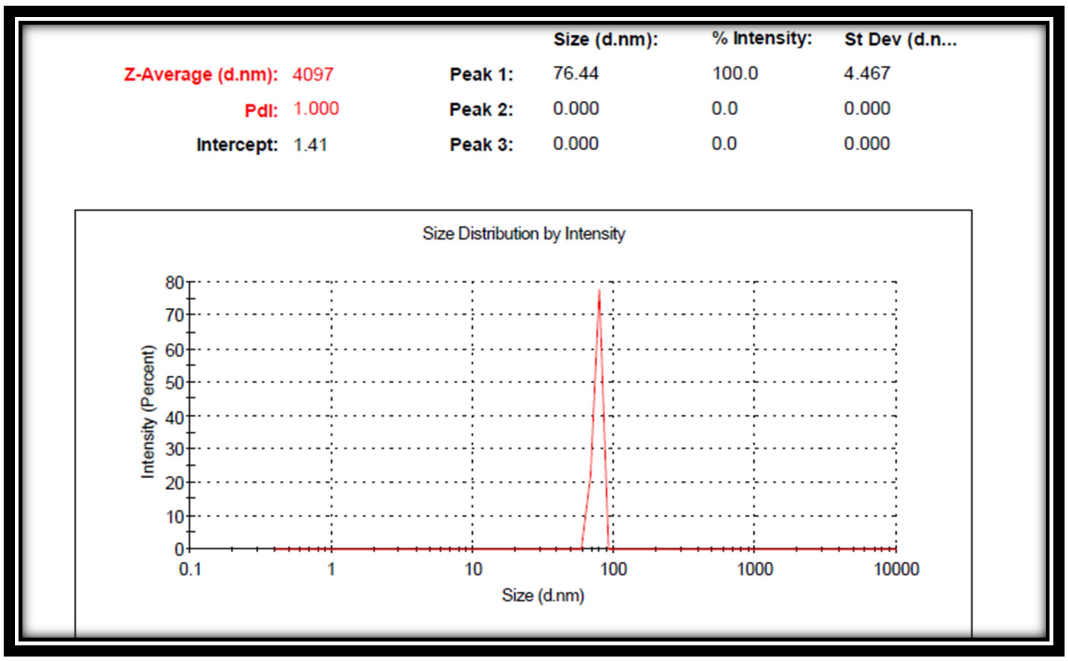


Figure 17: Zeta size distribution of Silymarin nanosponges (F4)

Particle size analysis showed that the average particle size of Silymarin nanosponges formulated using eudragit was found to be 3811nm with PDI value 1.000 and with intercept 1.33. The zeta size distribution of eudragit - Silymarin nanosponges is depicted in Figure 18.

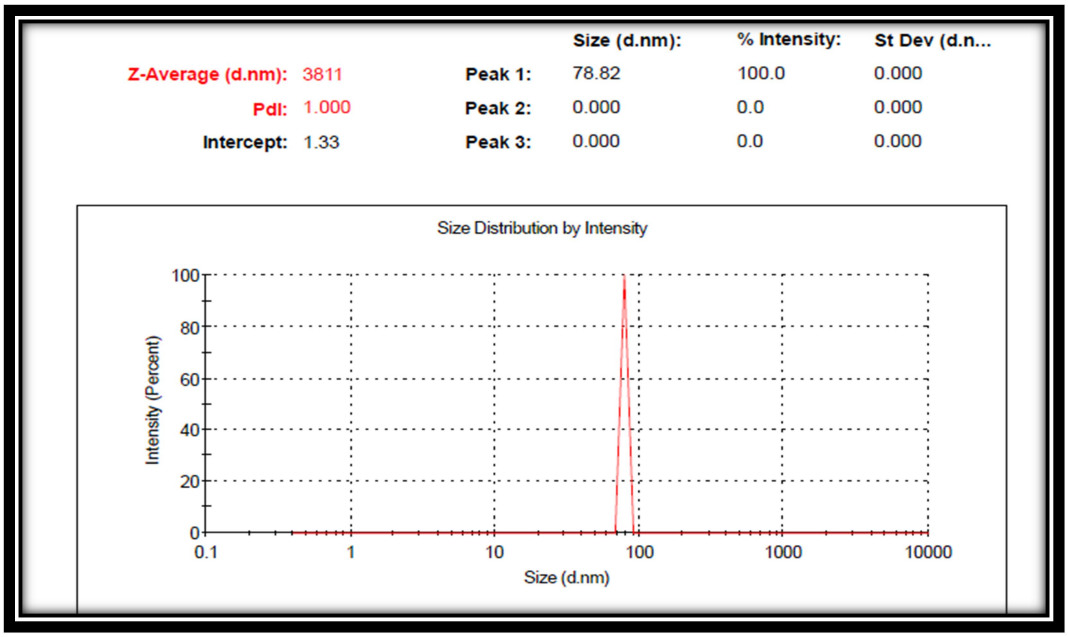


Figure 18: Zeta size distribution of Silymarin nanosponges (F9)

The particle size of nanosponges must be less than 5µm [1] **(**Ahmed R Z. *et al*.). The average particle size analyses of ethyl cellulose-Silymarin and eudragit-Silymarin nanosponges are 4097nm and 3811nm respectively which are lesser than 5µm.

Determination of Zeta Potential

Zeta Potential was determined using Malvern zeta-sizer instrument. Zeta potential analysis is carried out to find the surface charge of the particles to know its stability during storage. The magnitude of zeta potential is predictive of the colloidal stability. Nanoparticles with zeta potential value greater than +25 mV or less than -25 mV typically have high degrees of stability. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values then there will be no force to prevent the particles coming together and flocculating.

For Silymarin nanosponges using ethyl cellulose zeta potential was found to be -15.2 mV with peak area of 100% intensity. These values indicate that the formulated Silymarin nanosponges (F4) are stable. Zeta potential distribution of Silymarin nanosponges prepared using ethyl cellulose is depicted in the Figure 19.

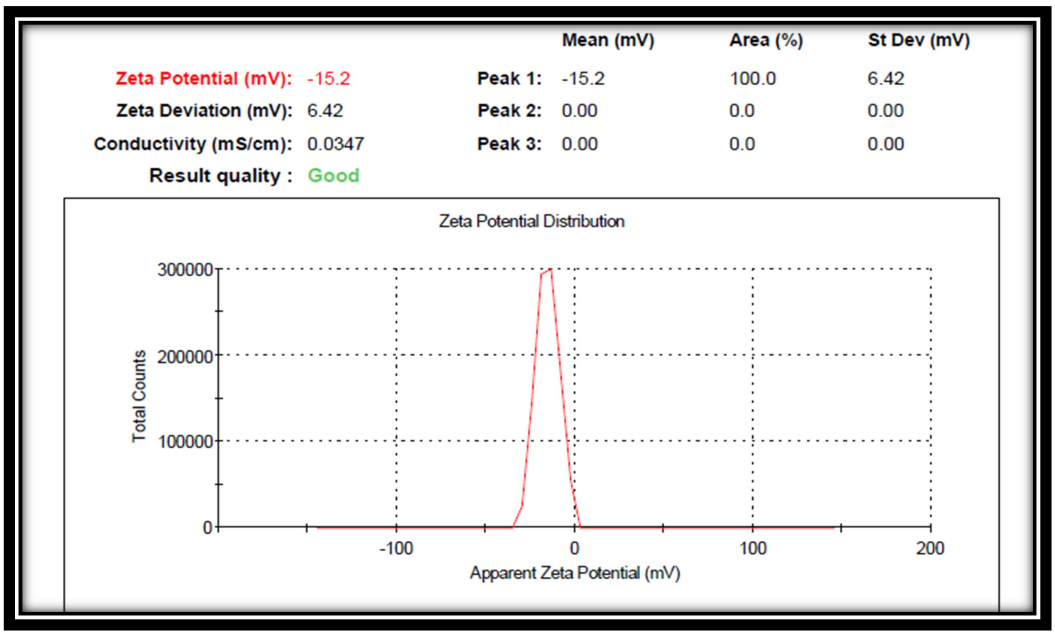


Figure 19: Zeta potential of Silymarin nanosponges (F4)

For silymarin nanosponges using eudragit zeta potential was found to be -23.4mV with peak area of 100% intensity. These values indicate that the formulated Silymarin nanosponges (F9) are stable. Zeta potential distribution of Silymarin nanosponges prepared using eudragit is depicted in Figure 20.

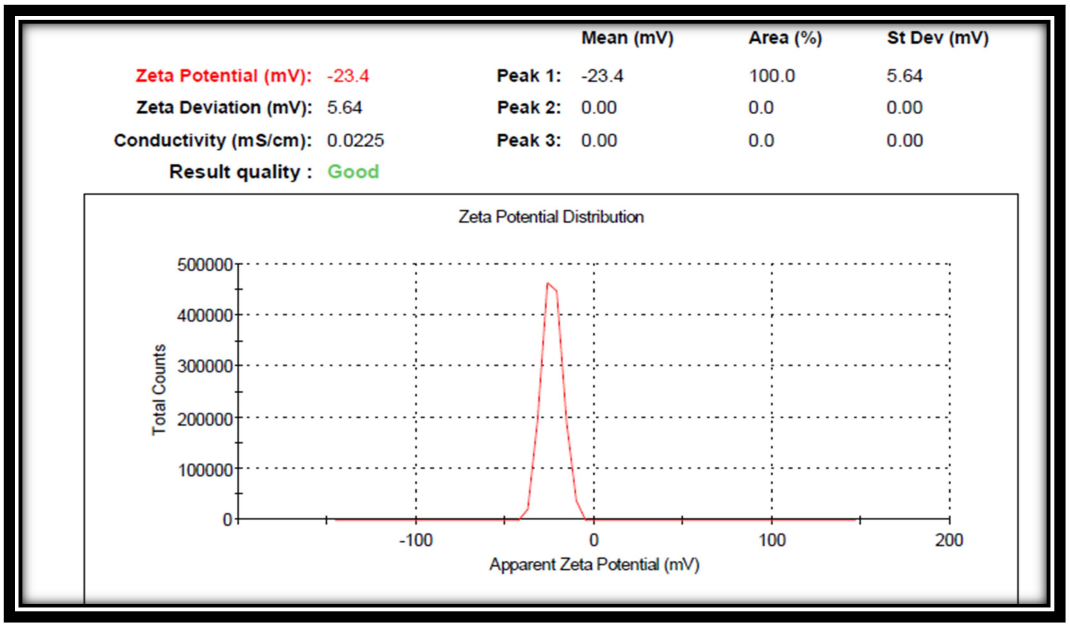


Figure 20: Zeta potential of Silymarin nanosponges (F9)

Entrapment efficiency:

The amount of entrapped drug was calculated from the equation:

$$\%\ Drug\ Entrpment = \frac{Practical\ drug\ content}{Theoretical\ drug\ content} \times 100$$

Entrapment efficiency of prepared formulation is given in Table 21 and Figure 21.

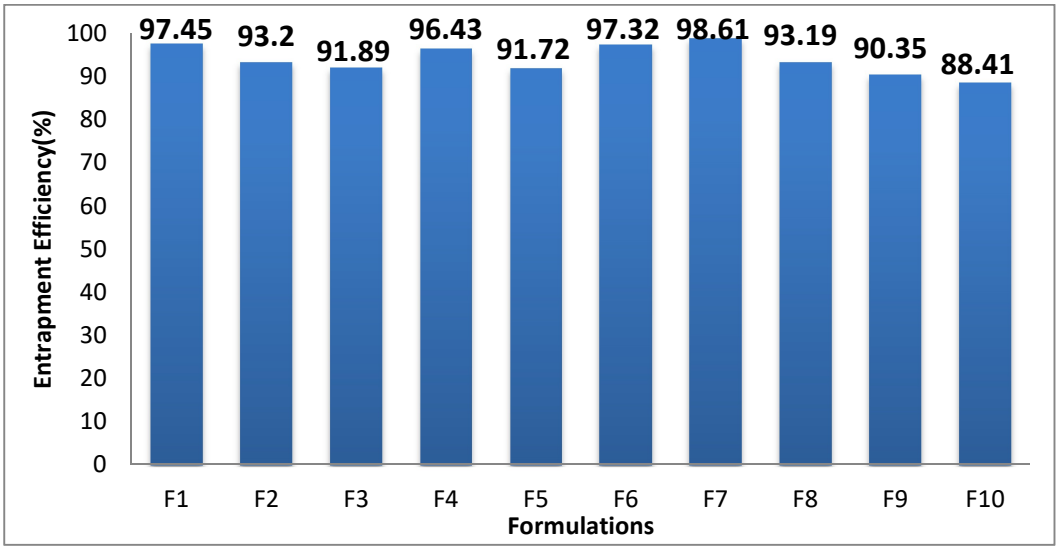


Figure 21: Entrapment efficiencies of Silymarin nanosponges

Table 21: Entrapment efficiencies of Silymarin nanosponges

S. No	Formulation code	Entrapment Efficiency (%)
1.	F1	97.45
2.	F2	96.43
3.	F3	91.89
4.	F4	93.20
5.	F5	91.72
6.	F6	97.32
7.	F7	98.61
8.	F8	93.19
9.	F9	90.35
10.	F10	88.41

The entrapment efficiency was found to be highest for F7 formulation (Silymarin: eudragit, ratio 1:1) which is 98.61% and the lowest entrapment of drug was found for F10 formulation (Silymarin: eudragit ratio 1:3). This might be due to the fact that the variation in entrapment efficiency was due to the changes in the polymer concentration and difference in the degree of cross linking (Viswanad *et al.*2015) [43]. The prepared nanosponges possess high drug entrapment efficiency and were found to be in the range of 88.41% - 98.61%.

IN VITRO DRUG RELEASE STUDIES

In vitro drug release study of the prepared Silymarin nanosponges was carried out using dialysis bag diffusion method. Amount of drug released in different time intervals were observed.

In vitro drug release profile data obtained of Silymarin nanosponges containing ethyl cellulose (F1-F5) are given in Table 22 and Figure 22.

Table 22: *In vitro* drug release profile of Silymarin nanosponges (F1-F5)

Sl. No	Time (hrs)	Cumulative percentage drug release (%)				
		F1	F2	F3	F4	F5
1	0	0	0	0	0	0
2	1	10.90	11.93	11.08	7.36	7.23
3	2	18.62	20.26	15.7	9.33	8.96
4	3	21.76	24.89	19.39	10.13	9.89
5	4	26.00	30.01	21.24	13.11	11.54
6	5	30.23	37.37	25.86	16.93	14.89
7	6	37.94	42.73	27.71	22.19	18.16
8	7	43.47	47.03	32.33	26.35	23.54
9	8	45.18	50.96	35.68	29.71	28.18
10	10	50.04	52.74	42.46	33.53	30.13
11	12	52.14	55.16	46.89	40.05	38.91
12	24	63.17	64.73	56.86	53.83	49.75
13	32	69.90	69.16	64.90	58.12	53.67
14	36	77.18	75.44	69.17	61.92	59.11
15	48	89.90	88.79	81.75	72.86	67.56

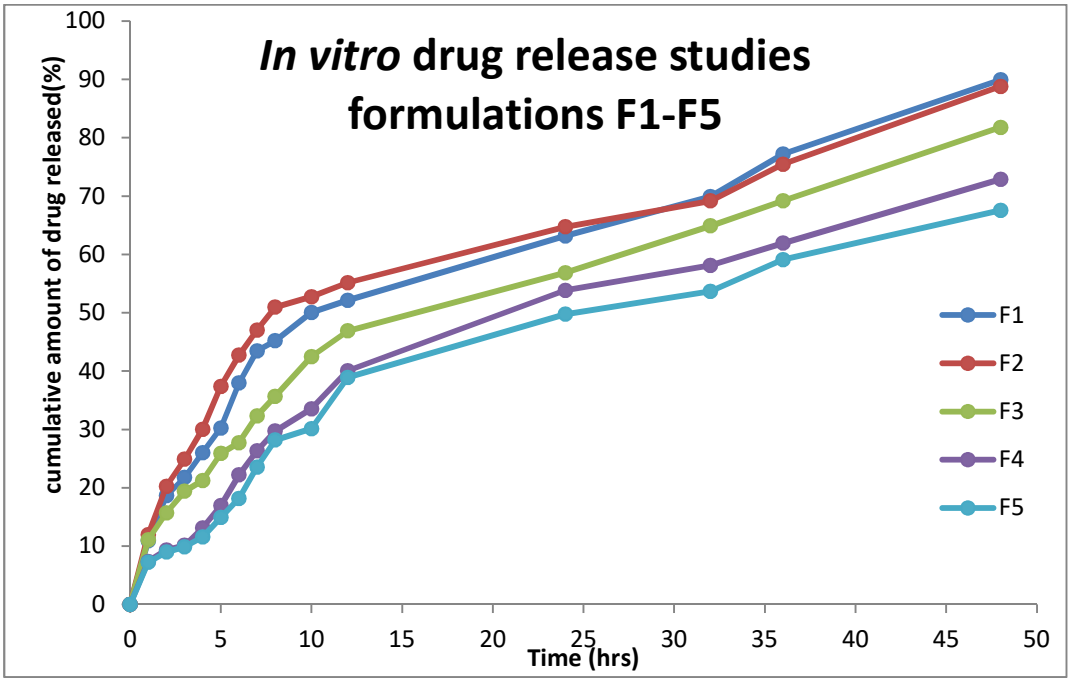


Figure 22: *In vitro* drug release profile of Silymarin nanosponges (F1-F5)

In vitro drug release profile data of Silymarin nanosponges containing eudragit (F6-F10) are given in Table 23 and Figure 23.

Table 23: *In vitro* drug release profile of Silymarin nanosponges (F6-F10)

Sl. No	Time (hrs)	Cumulative percentage drug release (%)				
		F6	F7	F8	F8	F10
1	0	0	0	0	0	0
2	1	13.44	14.32	14.06	8.99	7.45
3	2	16.48	18.35	17.77	10.27	9.06
4	3	22.39	22.14	22.26	11.30	10.87
5	4	27.18	27.04	24.41	13.10	12.12
6	5	31.4	30.05	29.05	13.87	15.68
7	6	36.16	34.24	32.02	16.44	18.86
8	7	41.64	41.08	36.57	20.55	24.98
9	8	45.19	43.61	39.09	23.76	29.12
10	10	51.4	49.35	43.43	36.99	32.19
11	12	54.16	53.67	48.13	40.18	39.16
12	24	62.41	62.53	55.89	48.91	50.80
13	32	70.85	68.51	61.24	55.16	54.89
14	36	76.18	73.27	66.75	61.19	60.23
15	48	90.18	87.10	77.94	70.14	69.86

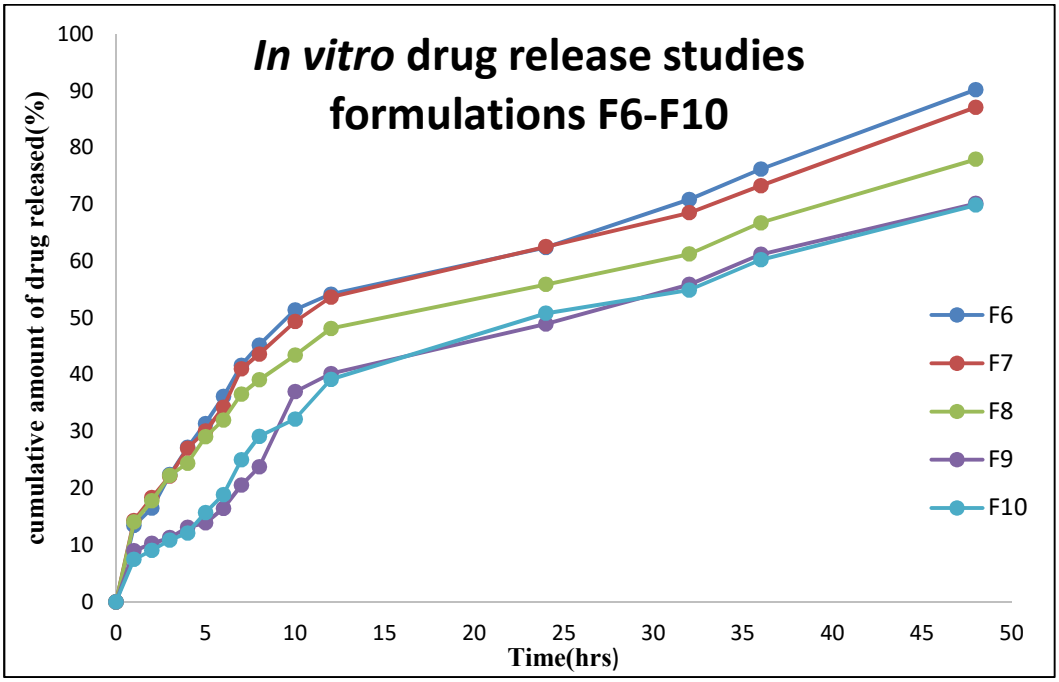


Figure 23: *In vitro* drug release profile of Silymarin nanosponges (F6-F10)

From the *in vitro* release data it was found that formulations F1 and F2 showed the best release of 89.90% and 88.79% respectively at the end of 48 hrs among all the five formulations of Silymarin - ethyl cellulose nanosponges. Similarly F6 and F7 exhibited the best release of 90.19% and 87.10% respectively at the end of 48 hrs among all the five formulations of Silymarin-eudragit nanosponges. The release rate was related to drug: polymer ratio. Increase of drug release was observed as a function of drug: polymer ratio. It was observed that the drug release decreased with an increase in the amount of polymer for each formulation. This may be due to the fact that the release of drug from the polymer matrix takes place after complete swelling of the polymer and as the amount of polymer in the formulation increases the time required to swell also increases. These results are in agreement with the release pattern of Silymarin nanoparticles observed by Hui-ping-sun *et al.* (2016) [25].

The newly developed nanosponges exhibit a core shell structure with a hydrophobic core formed by either ethyl cellulose(F1-F5) and eudragit(F6-F10) and a hydrophilic shell formed by PVA macromolecules. The release showed a bi-phasic pattern with an initial burst effect may be due to the unentrapped drug

adsorbed on the surface of the nanosponges. Drug release curve shows a faster release around 20% in the first few hours which can be attributed to release of drug molecule adsorbed on the surface of nanosponges, while remaining drug released for further few hours say around 7-8 hours may stem from drug molecule physically entrapped within hydrophilic outer shell. At the same time, hydrophilic PVA molecules that form the shell could also solubilise within aqueous medium and release part of drug. Remaining drug is probably entrapped within the core of nanosponges and are released in the later time period.

OPTIMIZATION OF SILYMARIN NANOSPONGES BY GENERAL FULL FACTORIAL DESIGN

The factorial design is a technique that allows identification of factors involved in a process and assesses their relative importance. Full factorial design is one of the best tools to declare the effect of different variables on the parameters of any formulation. In addition, any interaction between factors chosen can be identified. Construction of a factorial design involves the selection of parameters and the choice of response. Factorial design was the first degree model because they have maximum efficiency in estimating main effects. General full factorial design was created and analysed using Minitab software (Minitab 18 statistical software).

Independent variables polymer type (A) at two levels (eudragit and ethyl cellulose) and drug: polymer ratio (B) at five levels (1:0.5, 1:1, 1:1.5, 1:2 and 1:2.5) were used to find their influence on the dependent variable percentage drug released at the end of 48 hrs. The optimization of Silymarin nanosponges by general full factorial design is shown in Table 24 and Table 25.

Table 24: Factors for optimization by general full factorial design

Factors		Levels				
		1	**2**	**3**	**4**	**5**
Independent factors	Polymer type (A)	Ethyl cellulose	Eudragit	-	-	-
	Polymer concentration (B)	1:0.5	1:1	1:1.5	1:2	1:2.5
Dependent factors	% drug released at the end of 48 hrs.					

Table 25: Optimization of Silymarin nanosponges by full factorial design

Polymer type (A)	**Drug : Polymer Ratio (B)**	**Percentage drug released at 48th hr (%)**
Ethyl cellulose(A1)	1:0.5(B1)	89.90
Ethyl cellulose(A1)	1:1(B2)	88.79
Ethyl cellulose(A1)	1:1.5(B3)	81.75
Ethyl cellulose(A1)	1:2(B4)	72.86
Ethyl cellulose(A1)	1:2.5(B5)	67.56
Eudragit(A2)	1:0.5(B1)	90.18
Eudragit(A2)	1:1(B2)	87.10
Eudragit(A2)	1:1.5(B3)	77.94
Eudragit(A2)	1:2(B4)	70.14
Eudragit(A2)	1:2.5(B5)	69.86

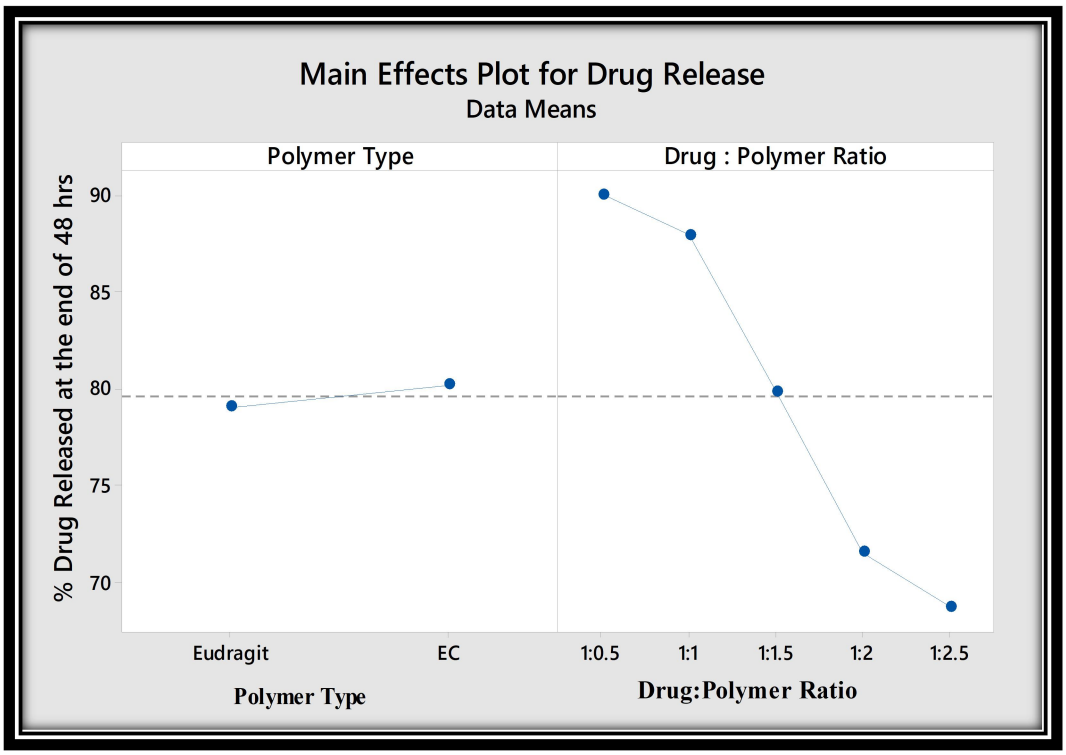


Figure 24: Main effects plot for drug release of Silymarin nanosponges

Effect of Polymer Type

Main effect plot of general full factorial design in Figure 24 reflected that the selected polymers has no much effect on the drug release pattern of Silymarin nanosponges. It may be due to the fact that both the polymers used (ethyl cellulose and eudragit) are hydrophobic in nature. The reason behind not selecting hydrophilic polymers in the formulation of nanosponges by emulsion solvent diffusion method was that they didn't produce promising results in trial batches. So selected hydrophobic polymers (EC and eudragit) used in the formulation of Silymarin nanosponges have no significant effect on the drug release pattern.

Effect of Drug: Polymer Ratio

Main effects plot of general full factorial design in Figure 24 reflected that the drug: polymer ratio influences the drug release pattern of Silymarin nanosponges. For both the selected polymers (EC and eudragit), as the drug: polymer increases the drug release decreases significantly. It is assumed that the possible mechanism behind the drug release is a combination of dissolution, diffusion and erosion. The

decrease in release pattern may be due to the difficulty of the drug to diffuse through the hydrophobic core.

IN-VITRO DRUG RELEASE KINETICS

The data obtained from the *in vitro* release study was used to fit into kinetic models. This was done to find out the mechanism of drug release from Silymarin nanosponges. In order to determine the release model, the *in vitro* release data were analysed according to zero order, first order and diffusion controlled mechanism according to simplified Higuchi model. The preference of a certain mechanism was based on the coefficient of determination (r^2) for the parameters studied, where the highest coefficient of determination is preferred for the selection of the order of release. The kinetic parameters of Silymarin nanosponges are shown in Table 26.

Since the r^2 value is higher for Higuchi model, it is selected as the best fitted model. This was confirmed by plotting percentage cumulative drug release and square root of time and r^2 value ranges between 0.8477 and 0.9888. This results are in agreement with results obtained by Srinivas *et al.*(2015) [45]. However, in many experimental situations, the mechanism of drug diffusion deviates from the Fickian equation and follows a non-Fickian (anomalous) behaviour. In these cases, the Korsemeyer–Peppas model was used to analyse the release kinetics. It is observed that formulation F1, F2, F6, F7 and F8 followed Fick's law of diffusion and rest showed an anomalous behaviour.

Table 26: Kinetic parameters of the release data of Silymarin nanosponges

Formula	r^2			Mechanism	Korsemeyer-Peppas Model		
	Zero order	First order	Higuchi		r^2	n	Mechanism
F1	0.4432	0.8976	0.9664	Diffusion	0.9768	0.441	Fickian
F2	0.1883	0.8307	0.9163	Diffusion	0.9637	0.386	Fickian
F3	0.5983	0.9069	0.9888	Diffusion	0.9892	0.487	Anomalous
F4	0.7729	0.9495	0.9674	Diffusion	0.9787	0.576	Anomalous
F5	0.7831	0.9401	0.9602	Diffusion	0.9742	0.585	Anomalous
F6	0.4323	0.8957	0.9652	Diffusion	0.9751	0.438	Fickian
F7	0.3948	0.8719	0.9638	Diffusion	0.9798	0.429	Fickian
F8	0.3404	0.7863	0.9620	Diffusion	0.9860	0.415	Fickian
F9	0.7910	0.9388	0.9489	Diffusion	0.9657	0.595	Anomalous
F10	0.7760	0.9412	0.9643	Diffusion	0.9765	0.579	Anomalous

Drug release kinetic models of formulations F1, F2, F3, F4, F5, F6, F7, F8, F9 and F10 are shown in figures 25,26,27,28,29,30,31,32,33 and 34 respectively.

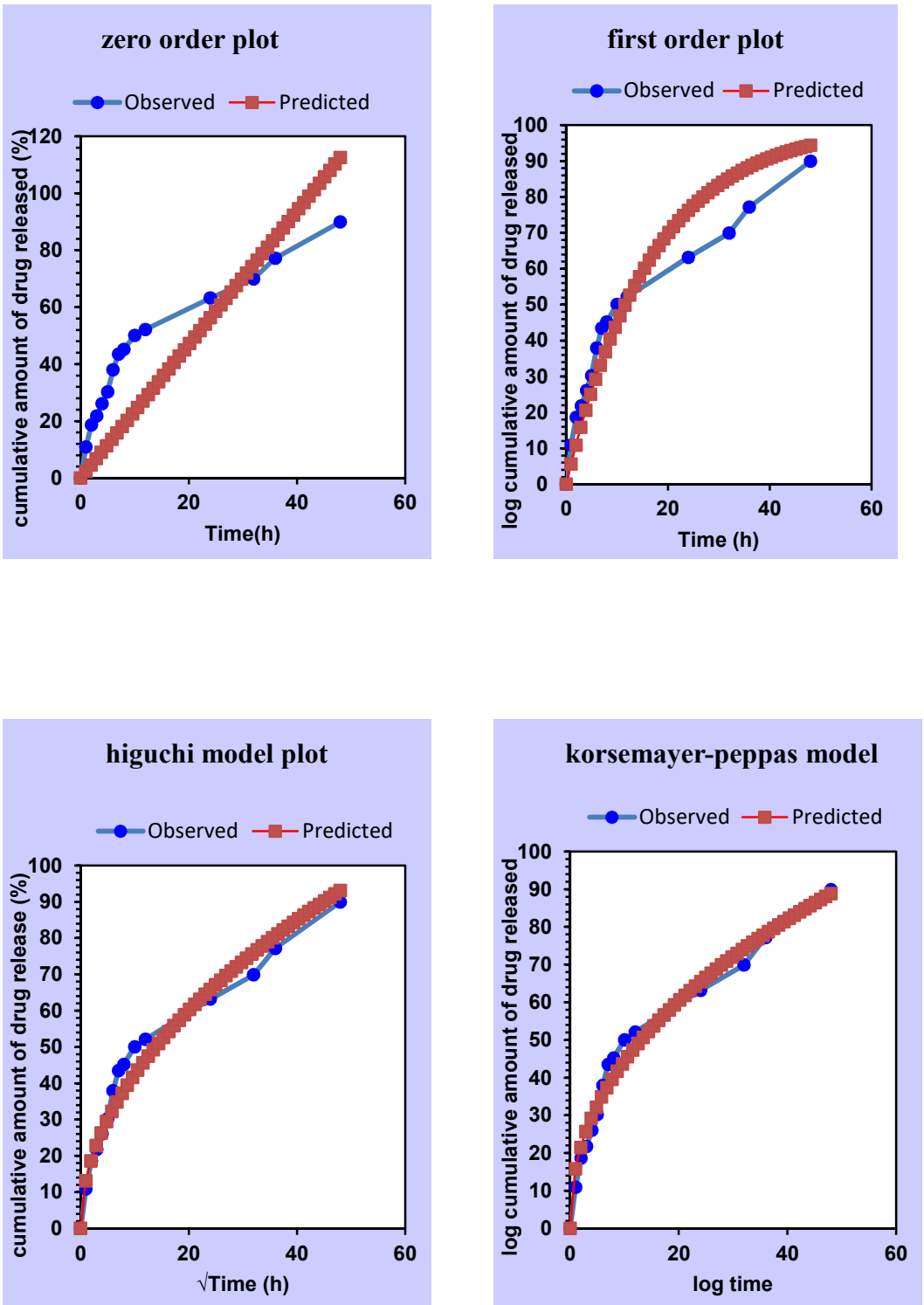


Figure 25: Drug release data of formulation F1 fitting to various kinetic models

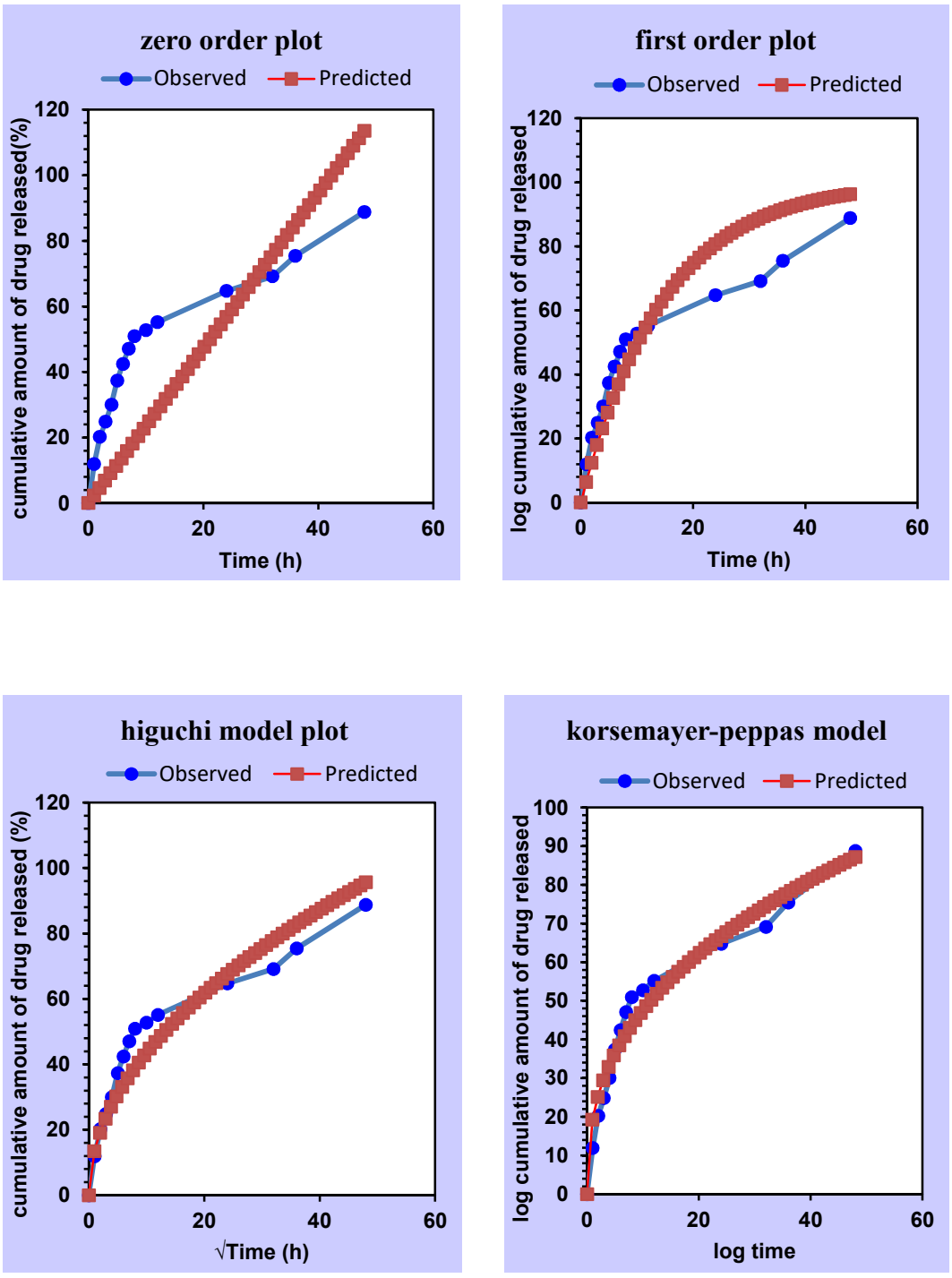


Figure 26: Drug release data of formulation F2 fitting to various kinetic models

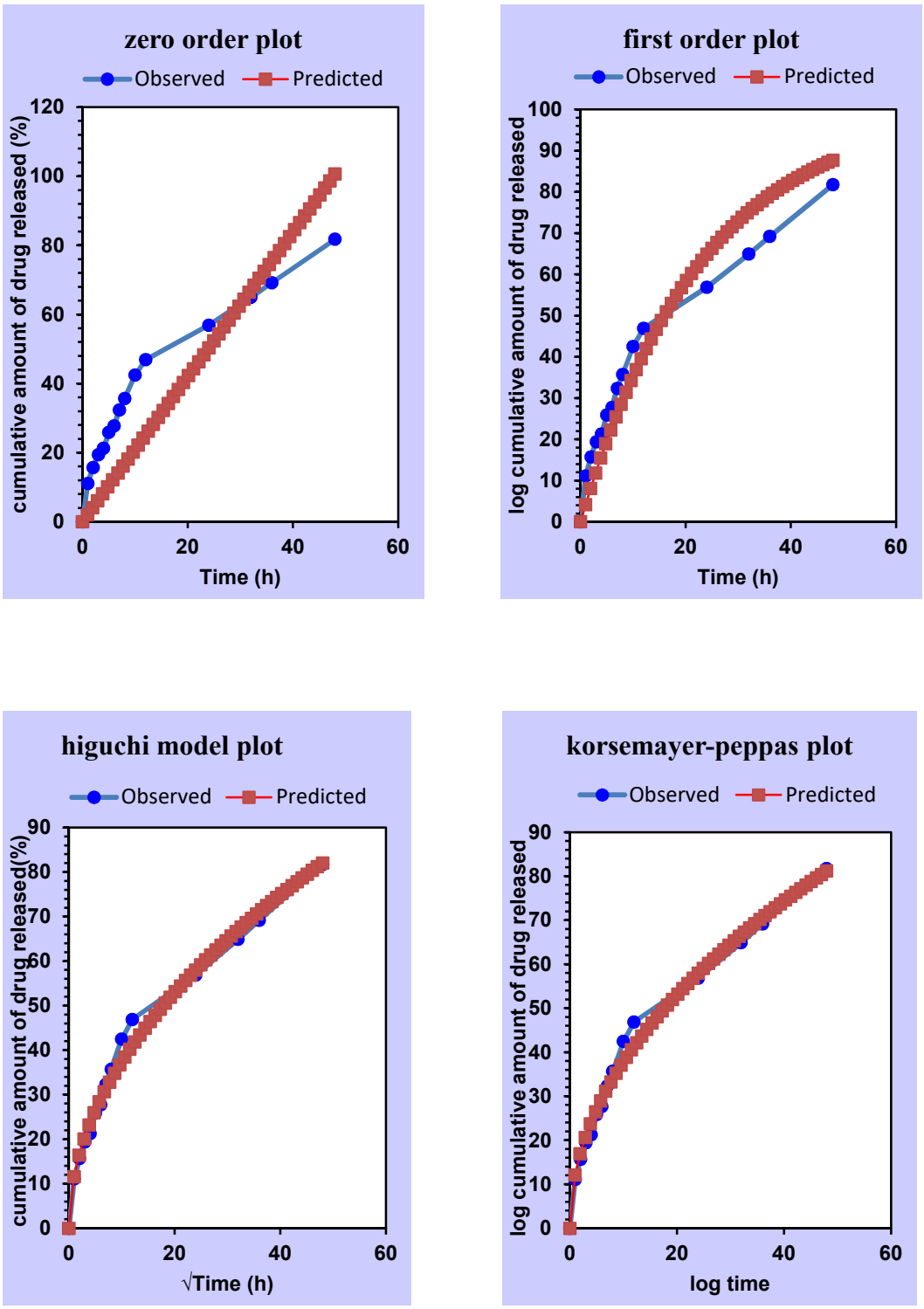


Figure 27: Drug release data of formulation F3 fitting to various kinetic models

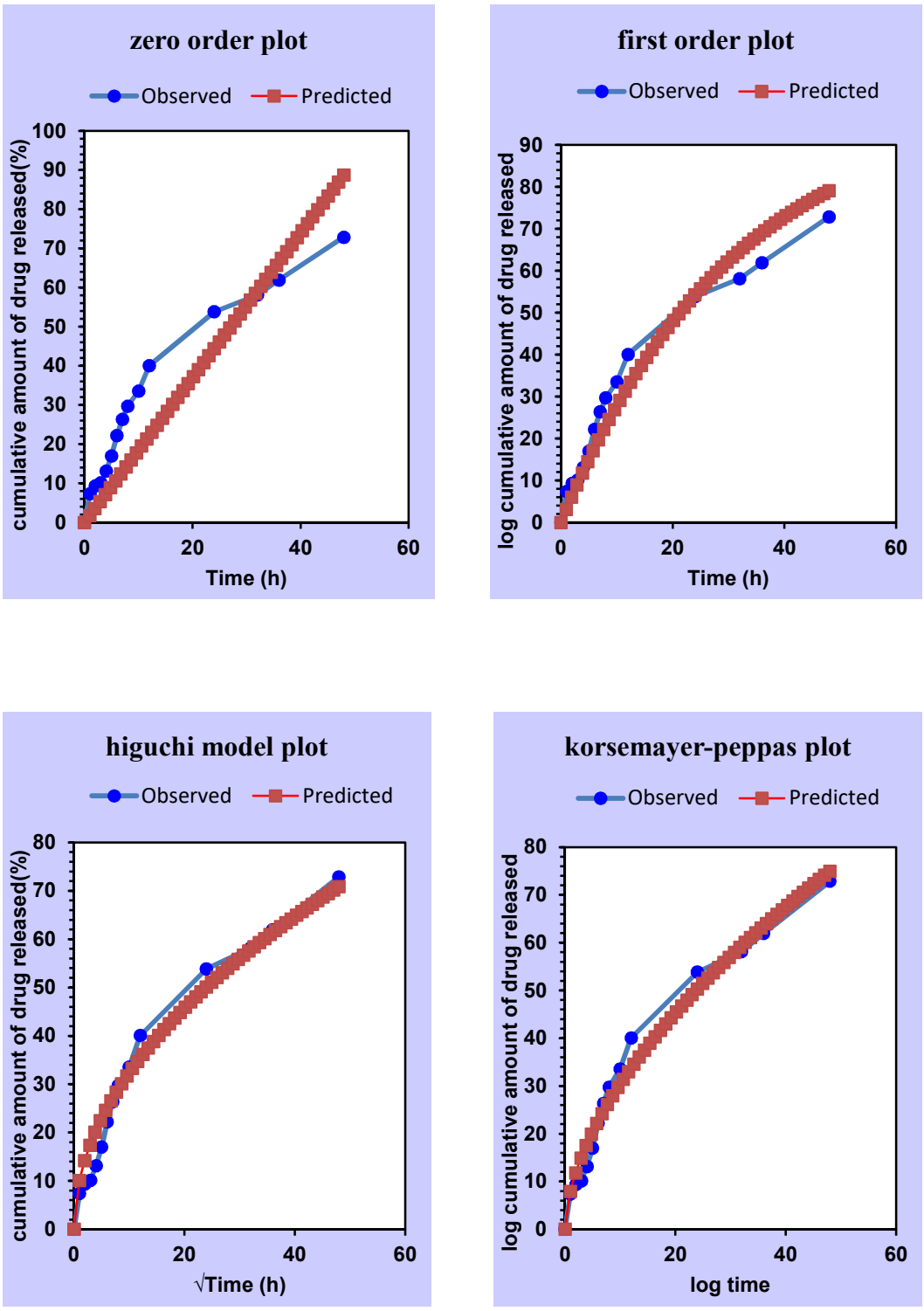


Figure 28: Drug release data of formulation F4 fitting to various kinetic models

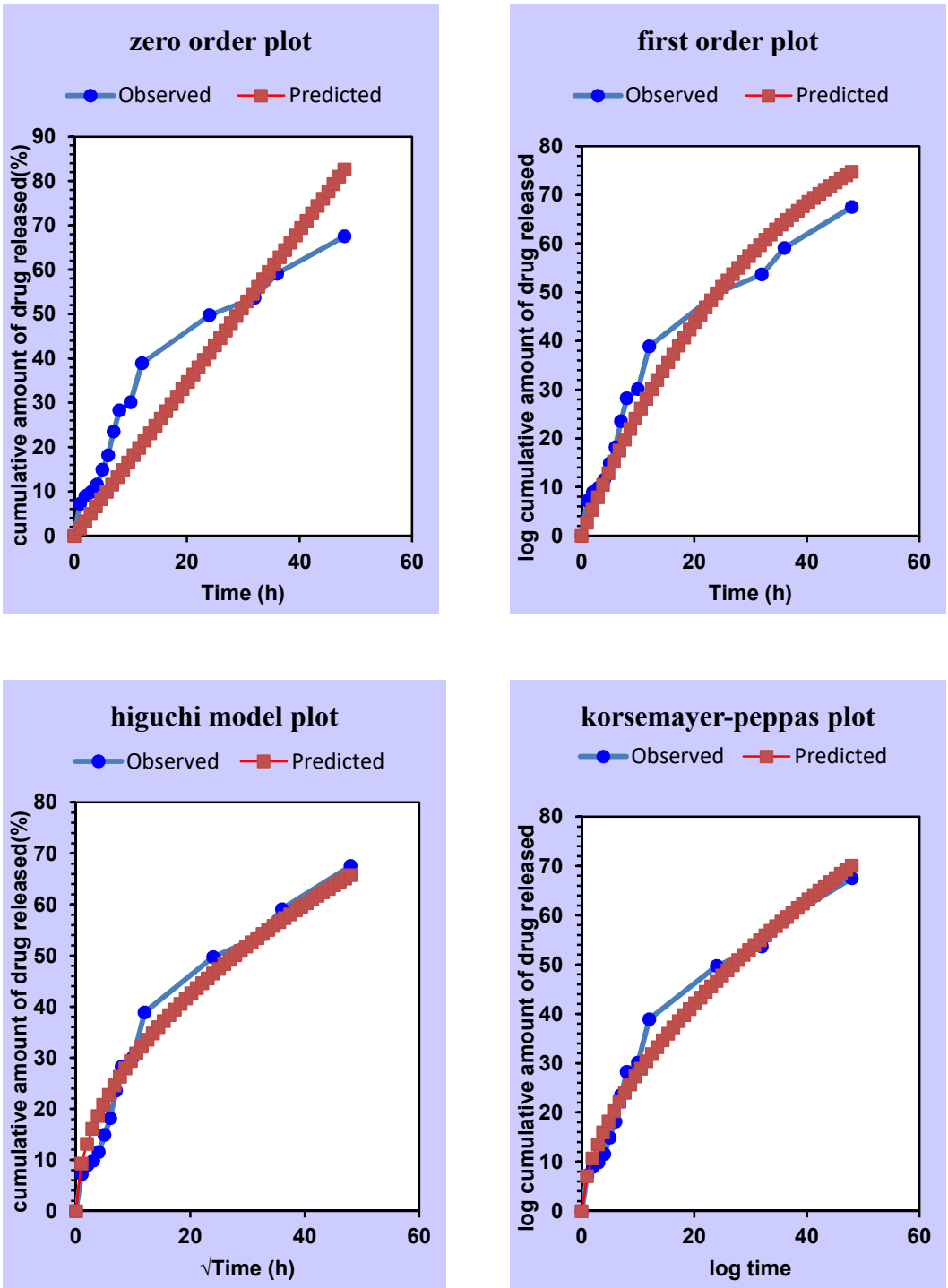


Figure 29: Drug release data of formulation F5 fitting to various kinetic models

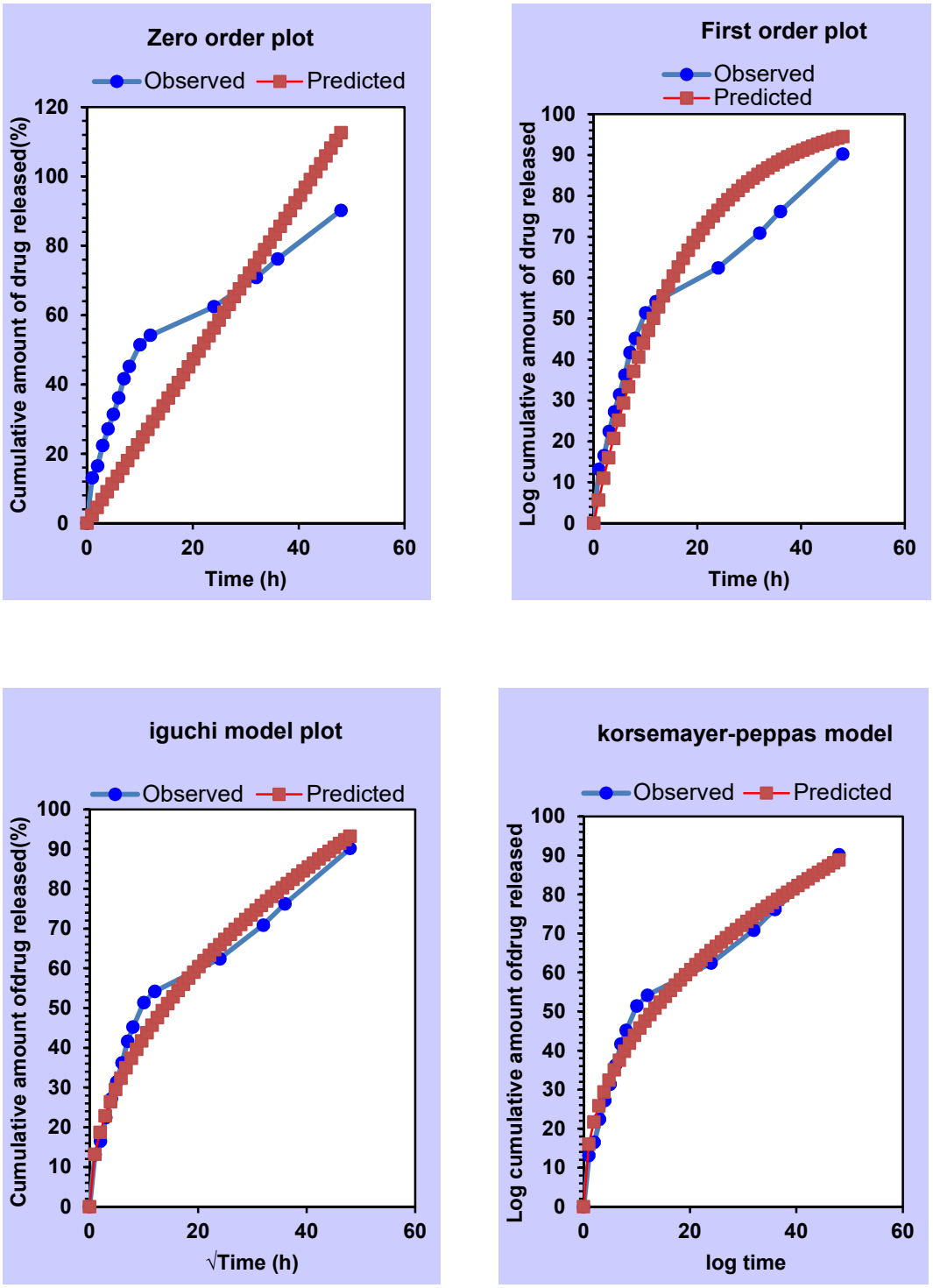


Figure 30: Drug release data of formulation F6 fitting to various kinetic models

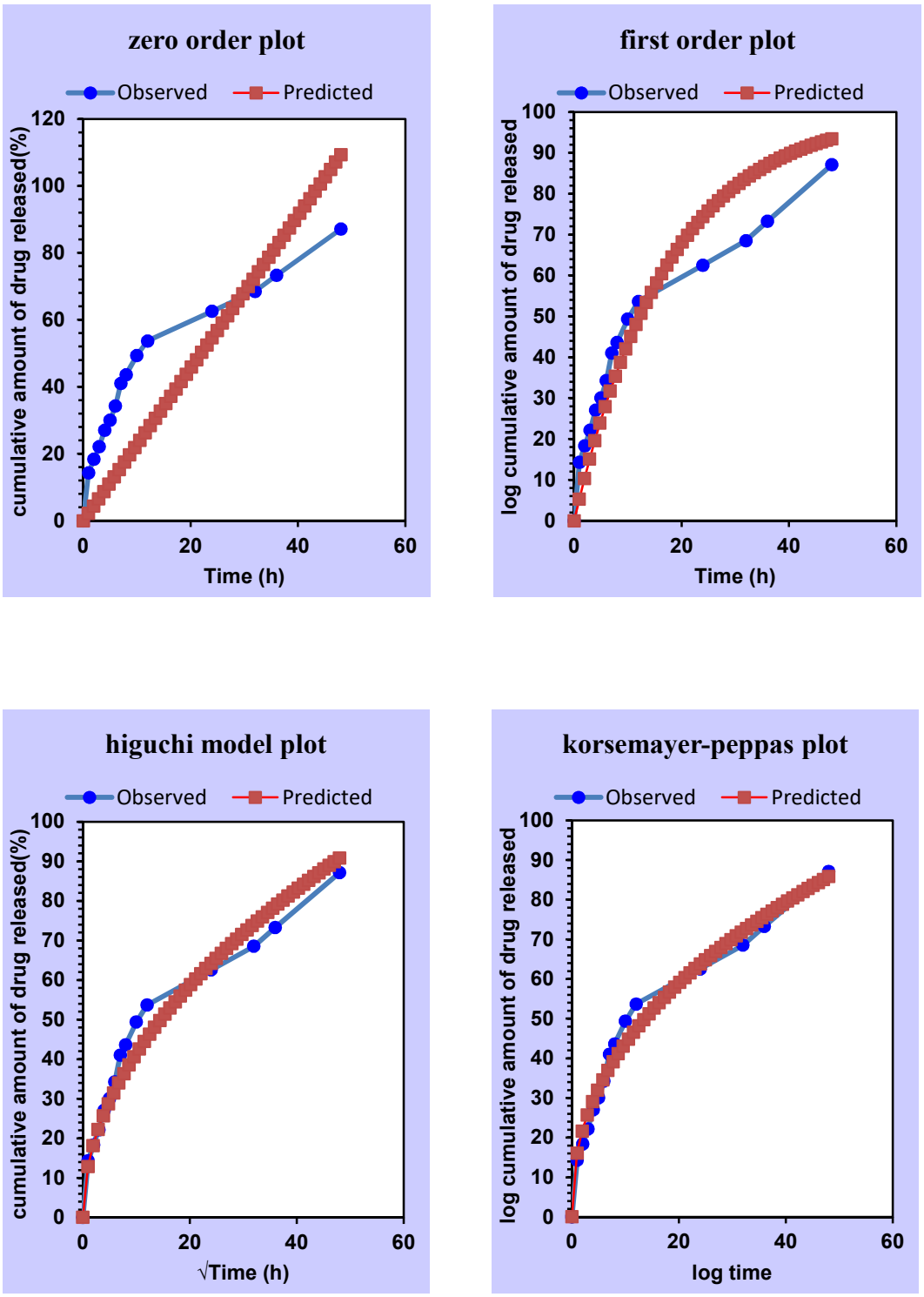


Figure31: Drug release data of formulation F7 fitting to various kinetic models

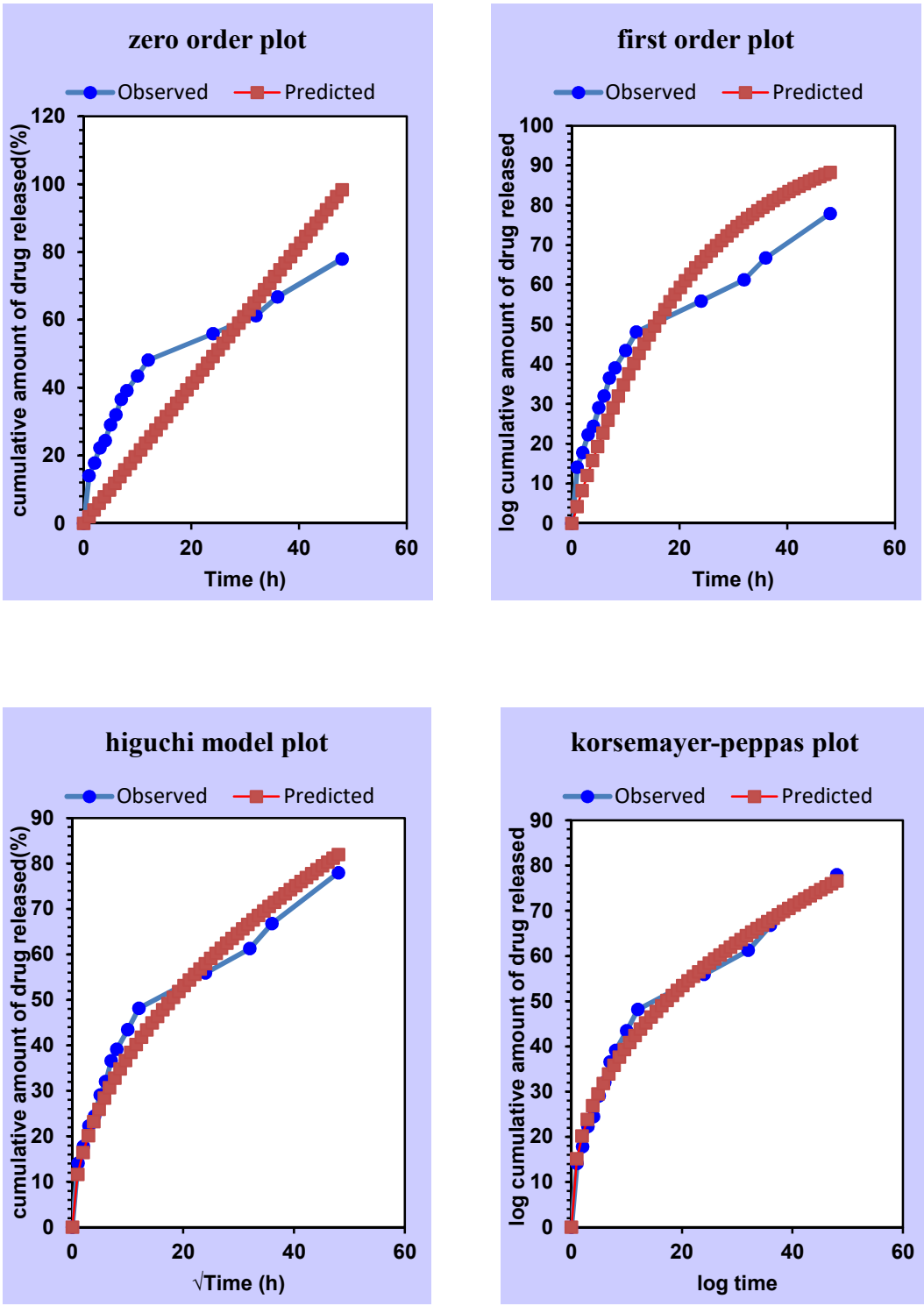


Figure 32: Drug release data of formulation F8 fitting to various kinetic models

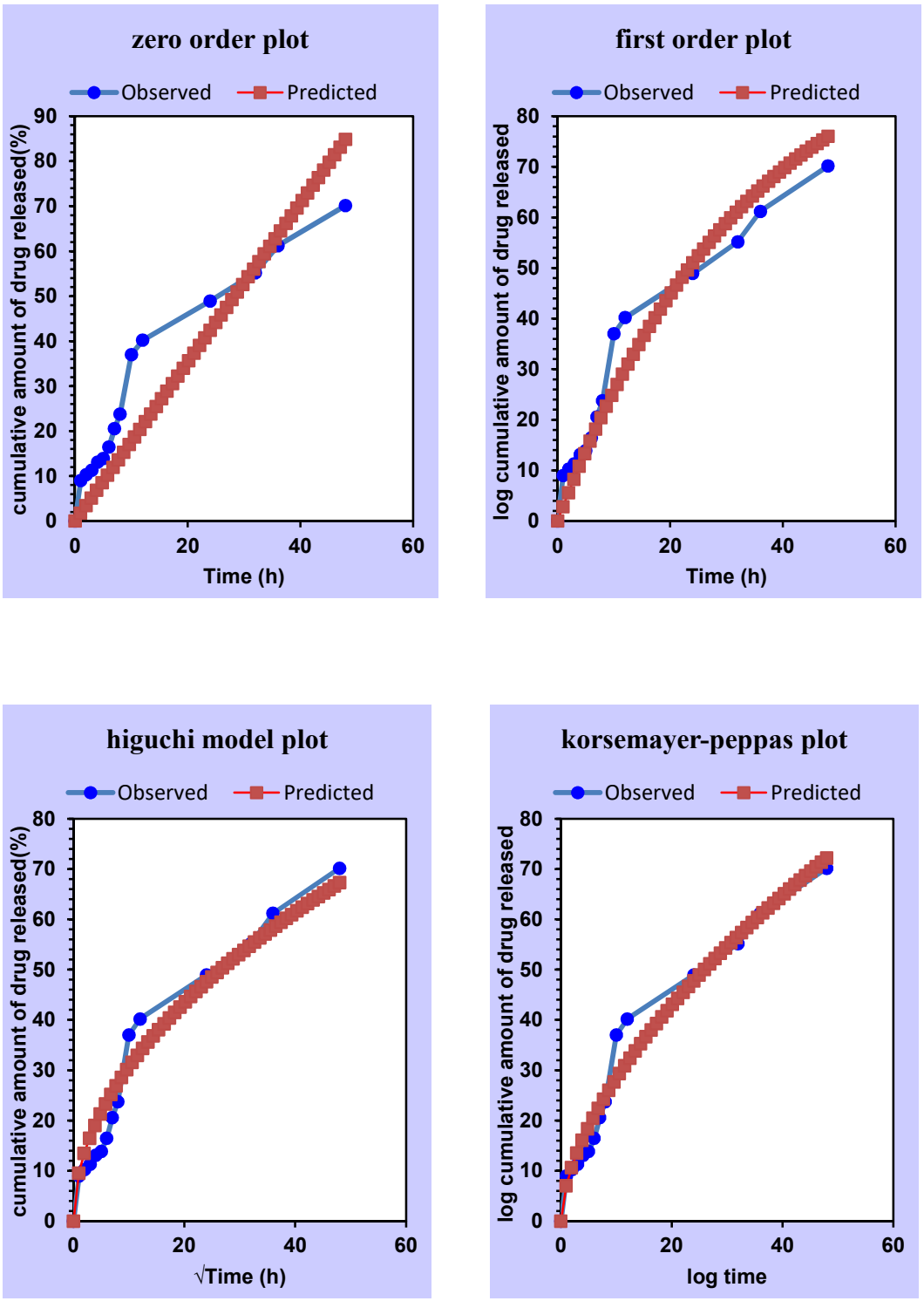


Figure 33: Drug release data of formulation F9 fitting to various kinetic models

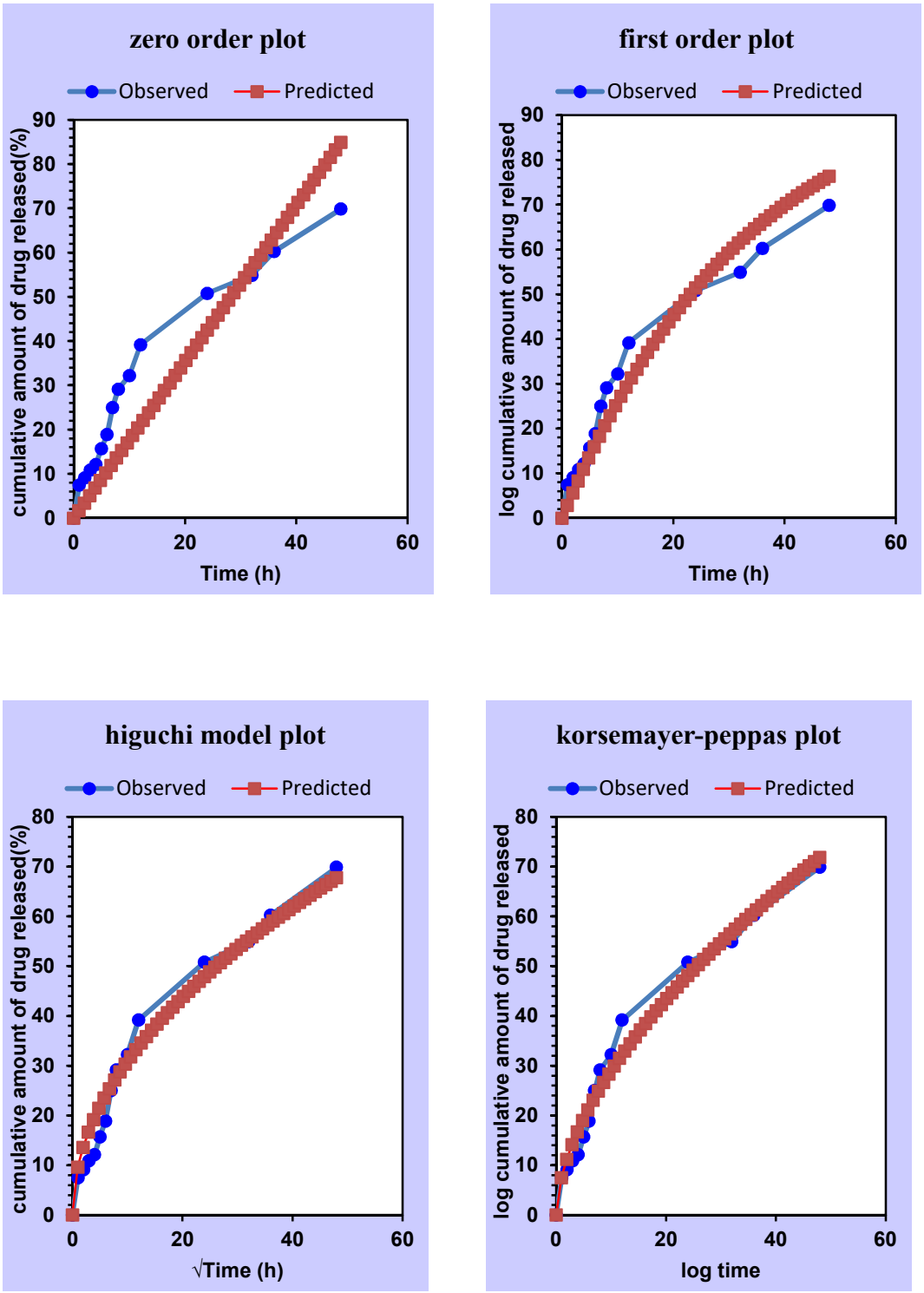


Figure 34: Drug release data of formulation F10 fitting to various kinetic models

SUMMARY AND CONCLUSION

Nanosponges are microscopic particles with few nanometers wide cavities, in which a large variety of substances can be encapsulated. These particles possess the ability to carry both lipophilic and hydrophilic substances and thereby improving the solubility of poorly water soluble molecules. Drugs encapsulated within the nanosponge pores are shielded from premature destruction and stability of drug is enhanced.

Silymarin, a potential phytochemical compound obtained from the seeds of *Silybum marianum* (milk thistle) plant has been used as a hepatoprotective agent, and shows strong anti-oxidant effect. Silymarin has been extensively studied *in vitro* and *in vivo* for its cancer chemopreventive potential against various cancers. The high incidence of administration of silymarin together with its short half-life and poor bioavailability proposed great scope for the proposal of nanoparticulate drug delivery systems. Various nanoformulation platforms employed as delivery vehicles for silibinin possess a major drawback in their low silibinin payloads.

Main objective of this study was to formulate Silymarin loaded nanosponges using different polymers to target cancer cells (breast cancer, colorectal cancer or oesophageal cancer) and release the drug in a controlled manner. This formulation reduced the side effects, minimized the dosing frequency and dose.

The present work aimed at formulating Silymarin nanosponges with two different types of polymers namely hydrophilic and hydrophobic polymers using emulsion solvent diffusion method. This method was simple and cost effective.

Preformulation studies were carried out to find out the solubility of Silymarin. Solubility test gave an idea that silymarin is not water soluble but soluble in solvents like acetone, dichloromethane etc.

FTIR and UV spectral studies authenticate the spectra obtained with the sample drug matched with standard pure drug. UV spectra gave the maximum absorption peak at 288nm.

The comparison of FTIR spectra of Silymarin and mixture of Silymarin and polymer confirms that there is no appearance of additional new peaks and

disappearance of existing peaks from that of the drug. This indicates that there is no interaction between the drug and polymer used in the study.

Formulation was carried out by emulsion solvent diffusion method. Trial batches indicated that hydrophilic polymers are not suitable for the Silymarin nanosponges. The hydrophilic polymers produced no yield or very less yield. Hydrophobic polymers produced good formulations. Ethyl cellulose and eudragit were selected for further studies.

Scanning electron micrograph of the prepared nanosponges at different magnification showed that the nanosponges were porous with a smooth surface morphology and spherical shape. The spongy and porous nature of nanosponges was clearly observed in the SEM images.

Particle size and zeta potential was determined by Malvern Zeta sizer. The particle size analysis confirmed that the prepared sample were in the nanometer range. Average particle size obtained for the formulations F4 and F9 were 4097nm and 3811nm. Zeta potential values of nanosponges indicated that the formulated nanosponges are stable.

The amount of drug being entrapped in nanosponges was calculated and all the prepared nanosponges were found to possess very high entrapment efficiency.

From the *in-vitro* release data from the dialysis bag diffusion method it was found that formulations F1, F2, F6 and F7 showed the best release of 89.90%, 88.79%, 90.19% and 87.10% respectively at the end of 48 hours. Increase of drug release was observed as a function of drug: polymer ratio. It was observed that the drug release decreased with an increase in the amount of polymer for each formulation. This is because the newly developed nanosponges is believed to exhibit a core shell structure with a hydrophobic core formed by ethyl cellulose(F1-F5) and eudragit(F6-F10) and a hydrophilic shell formed by PVA macromolecules.

The formulations were optimized by general full factorial design using Minitab 18 statistical software. Effect of polymer type and drug: polymer ratio on the drug release pattern of formulated Silymarin nanosponges on drug release was studied. It was found that the polymer type has no significant effect on the drug

release pattern whereas the drug: polymer ratio has a significant effect on the release pattern of the drug. As the drug: polymer ratio increases the release of drug from the nanosponges decreases. Even though the drug release from both Silymarin- ethyl cellulose nanosponges (F5) and Silymarin- eudragit nanosponges (F10) are similar, the F5 formulation is selected as the optimum formulation due to its comparatively higher yield.

The data obtained from the *in vitro* release study was fitted to the models which were used to find out the mechanism of drug release from silymarin nanosponges. The *in vitro* release model best fitted to Higuchi release order. This was confirmed by plotting percentage cumulative drug release and square root of time and r^2 value ranges between 0.8477 and 0.9888. It is observed that formulation F1, F2, F6, F7 and F8 followed Fick's law of diffusion and rest showed an anomalous behaviour.

The Silymarin nanosponges can be formulated by cost effective and easy emulsion solvent diffusion method using hydrophobic polymers such as ethyl cellulose and eudragit. The formulated silymarin nanosponges can be used in the treatment of cancer such as prostate or colorectal cancer. This can be targeted to the cancer cells and produce sustained drug delivery which in turn reduces the dose, frequency of administration and the side effects.

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